Molecular markers in benign and malignant soft tissue tumors

pathological and clinical implications

Uta Flucke

Molecular markers in benign and malignant soft tissue tumors

pathological and clinical implications

Proefschrift

Ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. Th.L.M. Engelen
volgens besluit van het college van decanen
in het openbaar te verdedigen op maandag 1 december 2014
om 12.30 uur precies

door

Uta Flucke
geboren op 16 augustus 1967
te Heiligenstadt, Duitsland

ISBN
978-94-6259-387-9

Lay-out
Promotie In Zicht, Arnhem

Print
Ipskamp Drukkers, Enschede

Promotoren

Prof. dr. J.H.J.M. van Krieken
Prof. dr. P.J. Slootweg
Prof. dr. W.T.A. van der Graaf

Manuscriptcommissie

Prof. dr. A.H.M. Geurts van Kessel
Prof. dr. P. Pauwels *(Universiteit Antwerpen, België)*
Prof. dr. J.H.W. de Wilt

Table of contents

Chapter 1

Introduction.
Soft tissue tumors: an overview and outline of the thesis

Soft tissue tumors are a highly heterogeneous group of tumors that are classified on a histogenetic basis according to the normal tissue they resemble.[1] This principle has been the basis of histopathological classifications published by pathologists with Arthur Purdy Stout being one of the most famous pioneers. He wrote the first AFIP Fascicle on soft tissue tumors in 1957.[2]

Soft tissue tumors are divided into a benign, intermediate and a malignant group. Most of the benign tumors more closely resemble normal tissue and have a limited capacity for autonomous growth, infiltration of adjacent structures and local recurrence following simple excision.[1] In contrast, malignant tumors, sarcomas, are locally aggressive tumors with a high tendency for recurrence and metastases.[3] They show varying resemblance to normal cell types, depending on their degree of differentiation.

Tumors of the intermediate group behave locally aggressive, but rarely metastasize.[4]

Finally, it should be noted that not all mesenchymal tumor types are classifiable on basis of a presumed line of differentiation from normal mesenchymal tissue such as ossifying fibromyxoid tumor, synovial sarcoma, epithelioid sarcoma, and alveolar soft part sarcoma, which have no normal cell counterpart.[1,4]

Classification

The most widely accepted classification is the WHO Classification of Tumours of Soft Tissue and Bone, of which the first edition, published in 1969 by Enzinger, included only soft tissue tumors while the current edition, published in 2013 by a large international team of pathologists, geneticists and medical oncologists, also includes lesions from bone.[4,5] This classification provides uniform and hence reproducible diagnostic criteria, which enables a more consistent approach to diagnosis, prediction of biological behavior as well as treatment. Furthermore, now that tumors are classified in a more consistent fashion, it will be easier to understand their intrinsic biology through identifying specific and consistent genetic aberrations.[5]

The WHO classification distinguishes between histogenetically defined groups: adipocytic tumors, (myo)fibroblastic tumors, fibrohistiocytic tumors, smooth muscle tumors, skeletal muscle tumors, pericytic tumors, vascular tumors, chondrosseous tumors, gastrointestinal stromal tumors, and nerve sheath tumors. Tumors without a normal cell-counterpart are subsumed in the group of tumors with uncertain differentiation.[4]

Histogenesis

Mesenchymal stem cells or multipotential precursor cells are self-renewing cells that can give rise to mesodermally derived tissues including fat, muscle, endothelium, connective tissue, tendon, bone, cartilage and stromal cells.[6,7]

According to the current understanding, most mesenchymal tumors are derived from these cells that are preprogrammed to differentiate into various mature cell

types as mentioned above. The preprogrammed nature of tumor stem cells explains why some sarcomas resemble their mature counterpart (eg. low grade leiomyosarcoma, well differentiated liposarcoma).[1] On the other hand, some sarcomas show a more primitive phenotype (e.g. Ewing sarcomas and alveolar rhabdomyosarcomas) closely resembling precursor cells and not always show a specific line of differentiation. The histogenesis of these and other sarcomas with no known normal cell counterparts could reflect a unique genetic makeup that has created peculiar tumor phenotypes.[1]

The origin of the stem cells or precursor cells is not clear, but it seems likely that many of them arise from the local, organ-specific pool of stem cells. In addition, data indicate that some soft tissue components are replenished from stem cells of bone marrow origin.[1,6,8]

Genetics

Tumors are genetic diseases with clonal expansion of neoplastic cells based on cancer-gene mutations playing a role in signal transduction pathways. The process of tumorigenesis is initiated when a replication-component (cell stem cell or partially differentiated descendent of a stem cell) acquires a mutation in a 'gatekeeping' pathway that endows it with a selective growth advantage.[9,10]

Increasing recognition of specific and/or recurrent genetic abnormalities in mesenchymal tumors and the growing use of genetic tests have aided in the definition of tumor types and the resolution of cellular origin.[1]

In cancer, alterations of oncogenes and tumor suppressor genes are quite common. Oncogenes achieve their effects through increased or deregulated activity of one of the two copies. This activation can be due to a mutation that alters the sequence of the protein with gain of function, increased copy number changes (amplification), or a translocation that brings its expression under the control of the regulatory sequences (promoter) of another gene, or to a combination of the above. In addition, translocations that fuse the coding sequences of two genes to generate chimeric proteins are generally considered a special variety of oncogenes, known as fusion oncogenes. There are numerous examples of these in soft tissue tumors, especially sarcomas.[3,11]

Tumor suppressor genes, in contrast, typically exert their oncogenic effects through a loss of both functional gene copies. This can be caused by one or more of the following mechanisms: a mutation that result in a truncated or inactive protein, mutations that result in a dominant negative protein that interferes with the function of the normal protein produced by the remaining unmutated gene copy, large deletions affecting the gene, replacement of the remaining unmutated copy (loss of heterozygosity by mitotic recombination), reduced expression due to hypermethylation of the gene's regulatory sequences, or interruption of the gene by a non-recurrent translocation.[3]

Several of the genetic alterations are of diagnostic and prognostic value, and the importance of molecular testing for guiding targeted therapeutic strategies in mesenchymal neoplasms is emerging.[1,11] In this approach, refered to as precision medicine, the goal is to develop cancer drugs matching specific molecular characteristics of a tumor, ultimately leading to: "The right drug, the right dose, for the right patient, at the right time".[12]

The aim of this thesis is to analyze specific genetic alterations in benign and malignant soft tissue tumors contributing to classification and improvement of diagnoses, especially in entities with overlapping features.

Outline of the thesis

In **chapter two**, we investigated *EWSR1* gene rearrangement in cutaneous myoepithelial tumors in order to compare them with their counterpart of soft tissue.
The issue of *EWSR1* gene rearrangement occurring in different benign and malignant tumors was further explored in **chapter three** and **four.**

In **chapter five**, we examined the diagnostic significance of genetic changes of *INI1* and the loss of the corresponding protein and its usefulness in the differential diagnosis between epithelioid sarcoma with prominent myxoid changes and myoepithelial tumors.

Chapter six pays attention to *C11orf95-MKL2* as a consistent fusion gene in chondroid lipomas and its role in diagnosis. This is an important issue as this rare and morphologically peculiar lipomatous lesion can be easily confused with malignant tumors such as extraskeletal myxoid chondrosarcoma and myxoid liposarcoma.

In chapter seven, we examined *RB1* in cellular angiofibromas. The morphologically suggested link with spindle cell lipomas and mammary-type myofibroblastomas as documented in the current WHO classification was also further analysed.

In **chapter eight**, desmoid fibromatosis of the head and neck in the children population is reported. These lesions are clinicopathologically not well-defined against other spindle cell lesions in the head and neck region and its molecular analysis was expected to be helpful.

References

1. Miettinen ed. Modern soft tissue pathology. Tumors and non-neoplastic conditions. Cambridge University Press, New York, NY, 2010.
2. Enzinger FM, Weiss SW. Soft tissue tumors. The C.V. Mosby Company, St. Louis, Missouri, 1983.
3. Weiss SM, Goldblum JR eds. Enzinger & Weiss's soft tissue tumors. 5th edition, Mosby Elsevier Philadelphia, PA, 2008.
4. Fletcher CD, Bridge JA, Hogendoorn PC, Mertens F eds. WHO classification of Tumours of soft tissue and bone.IARC, Lyon 2013.
5. Fletcher CD. The evolving classification of soft tissue tumours: an update based on the new WHO classification. Histopathology 2006:48:3-12.
6. Deans RJ, Mosely AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875-884.
7. Buehring HJ, Battula VL, Treml S, et al. Novel markers for the prospective isolation of human MSC. Ann NY Acad Sci 2007;1106:262-271.
8. Fletcher CDM ed. Diagnostic histopathology of tumors. 3rd edition, Elsevier, Churchill Livingstone, Philadelphia 2007.
9. Vogelstein B,Kinzler KW. Cancer genes and the pathways they control. Nature Medicine 2004;10:789-799.
10. Greaves M, Maley CC. Clonal evolution in cancer. Nature 2012;481:306-313.
11. Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S, Ladanyi M. Advances in sarcoma genomics and new therapeutic targets. Nat Rev Cancer 2011;11:541-557.
12. Stricker T, Catenacci DV, Seiwert TJ. Molecular profiling of cancer – the future of personalized cancer medicine: a primer on cancer biology and the tools necessary to bring molecular testing to the clinic. Semin Oncol 2011:38;173-185.

Chapter 2

EWSR1 gene rearrangement occurs in a subset of cutaneous myoepithelial tumors: a study of 18 cases

Mod Pathol 2011 Nov;24(11):1444-50.

Abstract

Cutaneous myoepithelial tumors form a clinicopathologic spectrum ranging from mixed tumor to myoepithelioma and myoepithelial carcinoma.

Recently, *EWSR1* rearrangement has been described in a subset of soft tissue myoepithelial tumors, whereas the cutaneous counterparts showed this aberration in a minority of cases. This raises the question whether cutaneous myoepithelial tumors have comparable genetic alterations.

We examined 18 cases of cutaneous myoepithelial tumors arising in seven female and eleven male patients (age range, 34-86 years; mean, 58 years). Eight mixed tumors occurred at the head, and one at the scrotum. Six myoepitheliomas arose at the extremities, and one case each at the back and head. One myoepithelial carcinoma occurred at the cheek. The tumor size ranged from 0.3 – 1.7 cm (mean, 1.0 cm). All mixed tumors and three myoepitheliomas were limited to the dermis. Four myoepitheliomas and the myoepithelial carcinoma involved the subcutis. Mixed tumors and myoepitheliomas were composed of myoepithelial cells with a variable cytomorphology, architecture and stromal background. Ductal structures were seen by definition in mixed tumors. The myoepithelial carcinoma represented an infiltrative dermal neoplasm consisting of atypical spindle cells.

Immunohistochemically, all cases tested were positive for EMA and calponin whereas S100, CK, ASMA and GFAP were expressed in 90%, 80%, 78% and 50% of the cases tested respectively.

By fluorescent *in situ* hybridization analysis, 7 out of 16 cases (44%) exhibited *EWSR1* rearrangement. Four of them were mixed tumors, two were myoepitheliomas and one was a myoepithelial carcinoma confirming that these lesions represent a spectrum of dermal myoepithelial tumors.

Follow-up information, available for five patients (including the patient with a myoepithelial carcinoma), revealed no evidence of disease in all cases (range, 6 – 72 month).

Our study provides a genetic relationship of myoepithelial tumors of the skin with their counterparts in soft tissue, bone and visceral localization by sharing *EWSR1* rearrangement.

Introduction

Cutaneous myoepithelial tumors form a continuous clinicopathologic spectrum ranging from benign mixed tumor to myoepithelioma and myoepithelial carcinoma. Although mixed tumors are not uncommon, purely myoepithelial tumors are very rare and only few and small series have been reported.[1-4]

The presence of preexisting myoepithelial cells in the skin and viscera (e.g. salivary glands, breast and lung) renders probably a pathogenetic difference with myoepithelial tumors of the soft tissue in which a normal counterpart does not exist.

As a consequence of the variability of myoepithelial cells, different morphologic patterns and a heterogeneous immunophenotype with alternating positivity for epithelial (keratins, EMA), myogenic (ASMA and calponin) and neurogenic markers (S100 and GFAP) occur.[1-6] Although criteria for malignancy in myoepithelial tumors of soft tissue have been established by moderate to severe nuclear atypia, the minimal criteria for malignancy among myoepithelial neoplasms of the skin remain uncertain.[4,6,7]

Hornick and Fletcher (2004) reported that increased mitotic activity (up to 6 mitoses/10 HPF) among cytologically benign cutaneous myoepitheliomas might predict recurrence and metastases.[4] Furthermore, it has been suggested that in the absence of malignant cytomorphology and confirmed metastatic disease, infiltrative margins, satellite tumor nodules, tumor necrosis and involvement of deep structures are ominous signs.[8] Fully malignant myoepitheliomas with malignant cytomorphology are known for their potential of aggressive behavior like their soft tissue counterparts.[6,9]

A recently published study showed that a subset of myoepithelial tumors especially in the soft tissues harbor *EWSR1* rearrangement with different fusion partners, and these genetic changes were reported in only two of six of cutaneous myoepithelial neoplasms.[10] Therefore it seemed appropriate to look for genetic similarities in a larger cohort of cutaneous myoepithelial tumors.

Material and Methods

The cases were retrieved from the (referral) files of 2 of the authors (TM, HK), and clinical details and follow-up were obtained from the referring physicians. In all cases, the tissue was fixed in 4% buffered formalin, routinely processed and embedded in paraffin; 2-4 µm thick sections were stained with hematoxylin and eosin and immunohistochemically by the labelled streptavidin biotin technique using commercially available antibodies listed in Table 1. Appropriate positive and negative controls were used throughout.

Table 1 Details of used immunohistochemical antibodies

Antibody	Clone	Dilution	Source
ASMA	1A4	1:500	DAKO
EMA	Mc5	1:400	BioGenex, San Ramon, USA
Pancytokeratin	MNF116	1:500	DAKO
S-100 protein	polyclonal	1:2000	DAKO
GFAP	GA-5	1:200	DCS, Hamburg
Calponin	CALP	1:400	DAKO, Glostrup, Denmark

Fluorescent *in situ* hybridization analysis

For the detection of a translocation involving the *EWSR1* gene on 22q12 and the *FUS* gene on 16p11 a directly FITC/Rhodamine-labeled break apart probe (Abbott, Bergisch Gladbach, Germany) was used.

Fluorescent *in situ* hybridization analysis (FISH) was performed on 3 µM sections of formalin-fixed, paraffin-embedded tissue after baking at 65°C for 16 h, deparaffinization with xylene and rehydration with ethanol. All tissue sections were pretreated with a 30% solution of Oncor pretreatment powder in 2xSSC and digested for 10 minutes with Proteinase K following the instructions of the suppliers (Q-Biogene, Heidelberg, Germany). After a second rehydration step, the probes were applied to the sections and the covered slides were sealed with rubber cement, heat-denatured and hybridized at 37°C for 16 h. All sections were counterstained with DAPI II in mounting medium (125 ng/ml, Abbott, Bergisch Gladbach, Germany) and visualized under a Zeiss Axioplan microscope using a HBO100 lamp and the appropriate filters for the three fluorescent dyes. A negative control has been used in each case. A case was considered having a break in case 10 of 50 counted tumor cells showed separation of a red and green signal.

Results

Eighteen cases were studied. Clinical data are shown in Table 2. Briefly, there were 7 females and 11 males with an age range of 34-86 years (mean, 58 years; median, 56 years).

Mixed tumors (n=9) occurred mostly on the head (n=8), whereas one was localized at the scrotum. Most of the myoepitheliomas (n=6) occurred at the extremities, one case each arose on the back and head. There was one myoepithelial carcinoma occurring at the cheek. The tumor size ranged from 0.3 – 1.7 cm (mean 1.0 cm).

Table 2 Clinical data and histological diagnosis

Case	Sex/ Age	Location	SC	size (cm)	diagnosis	RS	follow-up
1	m/34 y	occipital	-	1.0	mixed tumor	RM	NA
2	m/86 y	scrotum	-	1.2	mixed tumor	RM	NA
3	m/48 y	face	-	0.5	mixed tumor	R0	NA
4	m/55 y	nose	-	0.3	mixed tumor	RM	NA
5	m/72 y	frontoparietal	-	1.2	mixed tumor	RM	NED, 51
6	m/57 y	temple	-	0.5	mixed tumor	R0	NED, 12
7	f/61 y	parietal	-	1.7	mixed tumor	R1	NA
8	f/54y	face	-	0.4	mixed tumor	R0	NA
9	m/68 y	face	-	0.8	mixed tumor	R0	NA
10	f/51 y	back	+	1.5	myoepithelioma, rec	RM	NA
11	m/55 y	distal lower leg	+	1.2	myoepithelioma	R0	NED, 6
12	m/50 y	ear	-	1.5	myoepithelioma	R1	NA
13	f/65 y	arm	-	1.5	myoepithelioma	R0	NED, 17
14	m/66 y	thumb	+	1.5	myoepithelioma	RM	NA
15	f/49 y	finger	-	0.5	myoepithelioma	R0	NA
16	f/45 y	thigh	+	0.7	myoepithelioma	R0	NA
17	m/57 y	plantar	NA	NA	myoepithelioma	R1	NA
18	f/70 y	cheek	+	0.8	myoepithelial ca	R0	NED, 72

SC, subcutaneous; RS, resection status; NA, not available
R0, complete resection; RM, marginal resection; R1, microscopically positive resection margins; NED, no evidence of disease; rec, recurrence; ca, carcinoma

Mixed tumors were smaller than myoepitheliomas (0.9 cm vs. 1.2 cm). All mixed tumors and three myoepitheliomas were limited to the dermis; four myoepitheliomas showed involvement of the subcutis. In one myoepithelioma, the depth was not known. The myoepithelial carcinoma infiltrated the subcutis (Table 2).

Histologically, all mixed tumors were well circumscribed; seven had a nodular and two a nodular cystic architecture (Figure 1). Ductal structures were present by definition in all cases (Figure 2), sometimes cystic dilated and sometimes with apocrine features (n=3). Cribriform structures were present in one case. The myoepithelial component was arranged around ducts and also in nests, cords and strands, solid and reticular. Although epithelioid myoepithelial cells occurred in all cases, spindle cells were present in three cases and plasmocytoid cells in five cases. One case showed

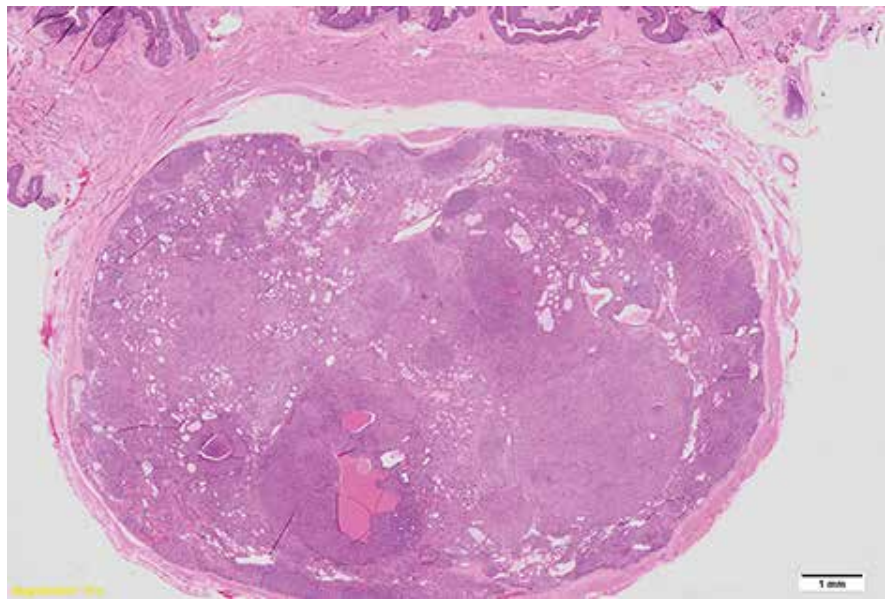

Figure 1 All mixed tumors had a nodular architecture (Case 2)

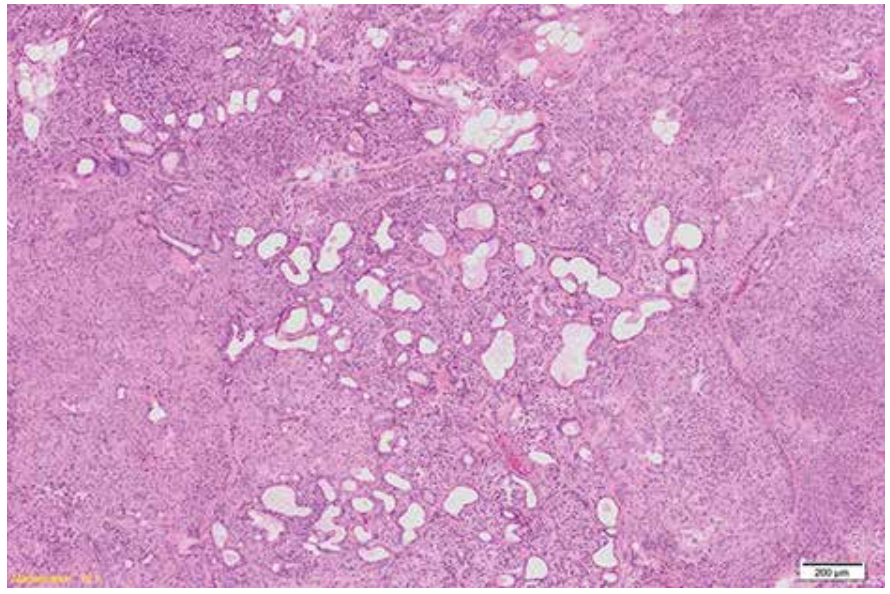

Figure 2 Glandular structures and a varying number of myoepithelial cells were present in all mixed tumors (Case 2)

focally clear cells. Metaplastic fat cells were a feature in Cases 1, 2, and 7 and squamous metaplasia in Cases 1 and 6. Ischemic central necrosis was seen in Case 5. No case showed mitotic figures and in Case 3 focal moderate atypia was noted. The stroma was myxoid at least focally in all cases, chondroid in four cases, hyaline and/or collagenous in five and three cases, respectively.

Three of the myoepitheliomas displayed a multinodular and three a nodular pattern, one of them with an epidermal collarette (Figure 3). A nodular-cystic appearance was noted in one case. One neoplasm was not sharply demarcated.

In each myepithelioma, different cell types were present. Two tumors contained mainly spindle cells and some epithelioid cells mostly arranged in bundles (Figure 4). Two tumors with a nested pattern had a predominantly plasmocytoid/epithelioid composition. In one of them double nuclei were present as seen in plasma cells. Epithelioid and spindle cells were observed in four cases accompanied by clear cells in Case 10 and plasmocytoid cells in Case 12. Strands, cords and nests were found in the latter cases (Figure 5). The matrix was at least focally myxoid in five cases, collagenous/hyaline in five cases, and focally chondroid in one case. Adipocytic differentiation occurred in one case. Little atypia was present in Case 11 and moderate atypia in Case 13 (Figure 6). The last one and Case 14 had 1 mitotic figure per 10 HPF, whereas in all other cases mitoses were absent.

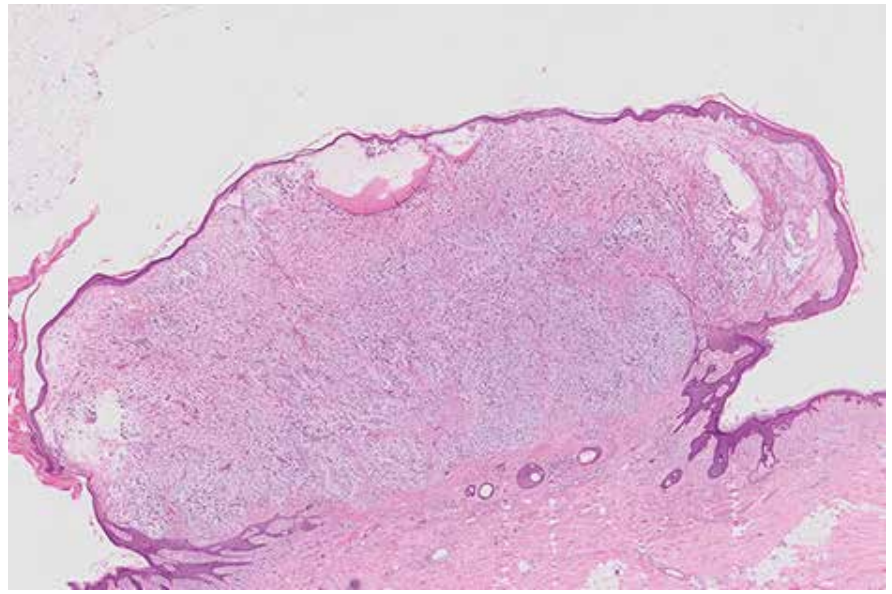

Figure 3 Case 13 was an exophytic lesion with an epidermal collarette

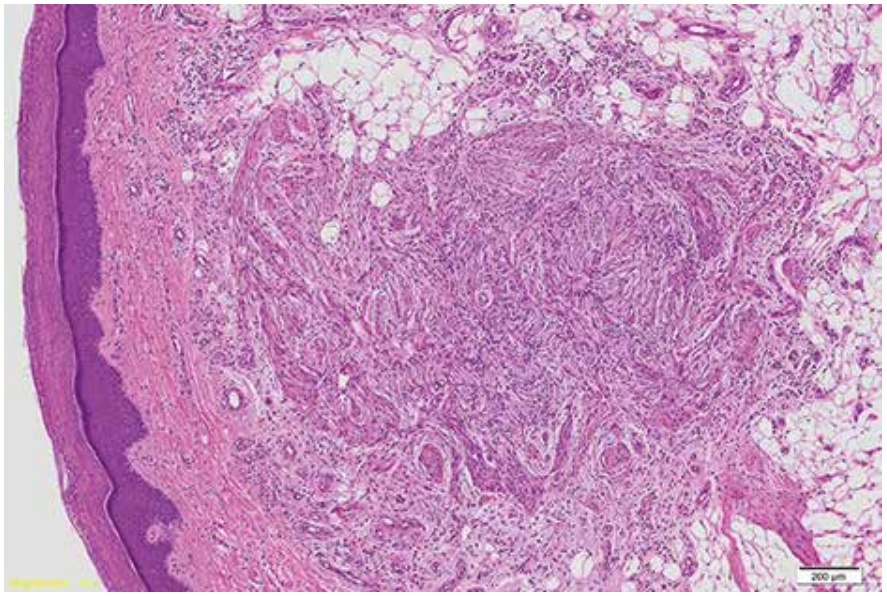

Figure 4 Case 11 showed predominantly spindle-shaped myoepithelial cells and also metaplastic adipocytes

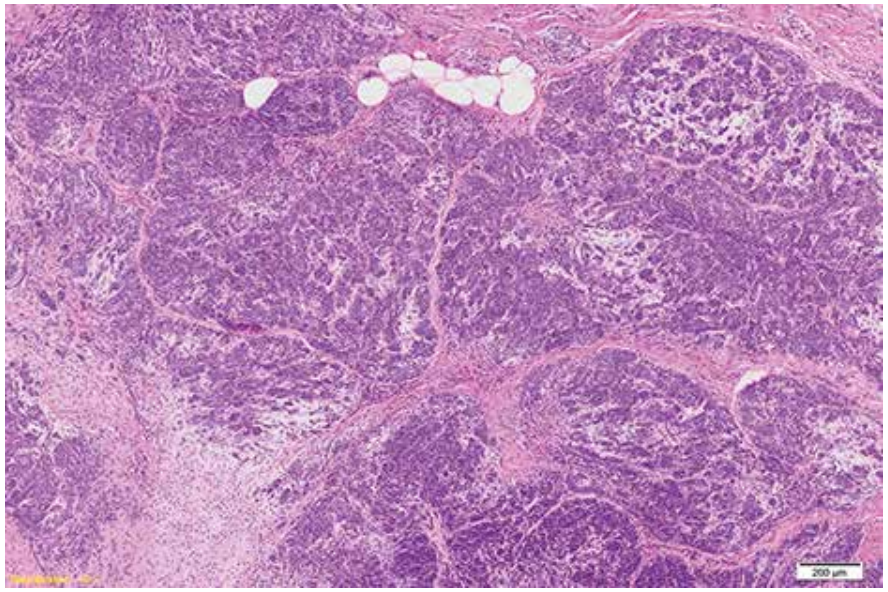

Figure 5 Case 10 was mainly composed of epithelioid cells with a predominantly nested growth pattern. Note the chondromyxoid stroma

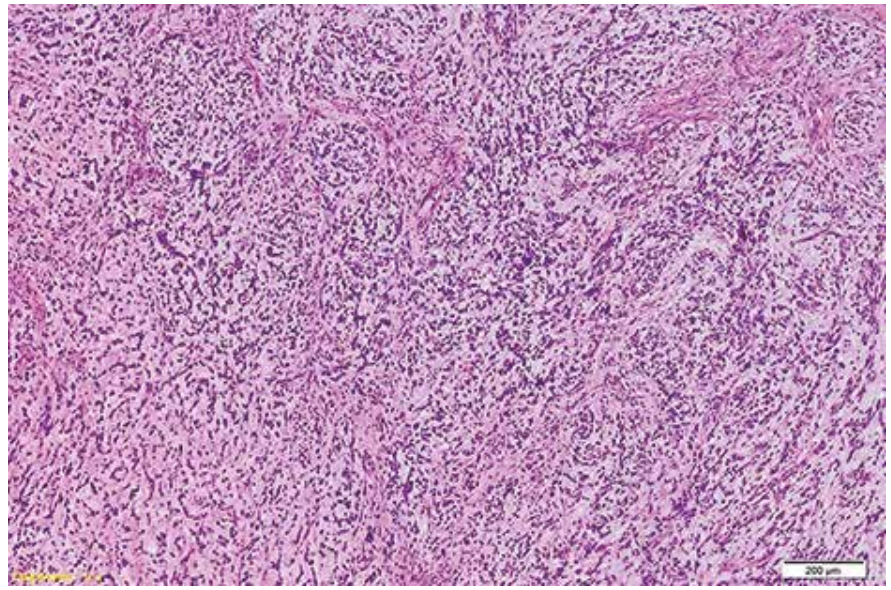

Figure 6 Moderate atypia was seen in Case 13. Note the reticular architecture and the myxoid matrix

The myoepithelial carcinoma (Case 18) was described earlier by the authors (Mentzel et al. 2003) and represented an infiltrating neoplasm composed of atypical spindle cells with enlarged and pleomorphic nuclei containing prominent nucleoli. There were 5 mitoses per 10 HPF. Focal tumor necrosis was present (Figure 7).

Immunohistochemical results are shown in Table 3. All cases tested were positive for EMA and calponin whereas S100, CK, ASMA and GFAP were expressed in 90%, 80%, 78% and 50% of the cases tested, respectively (Figure 8 and 9).

By FISH analysis, 7 out of 16 cases (44%) exhibited *EWSR1* rearrangement. Four of them were mixed tumors, two were myoepitheliomas and one was a myoepithelial carcinoma (Figure 10). Separation of the signal was generally seen in at least 20% of the nuclei. There were no morphological differences between positive and negative cases.

FUS gene rearrangement examined in the *EWSR1* negative instances was not detected in any of them (n=9) (Table 4).

In nine cases, the tumors were excised with wide tumor-free margins, and in six cases, the tumor were marginally excised. Microscopically tumor positive margins were observed in three cases (Table 2).

Follow-up data were available in five patients (two with myoepitheliomas, two with mixed tumors, one with a myoepithelial carcinoma) without evidence of disease during a follow-up time with a range of 6 – 72 month (mean, 32 month) (Table 2).

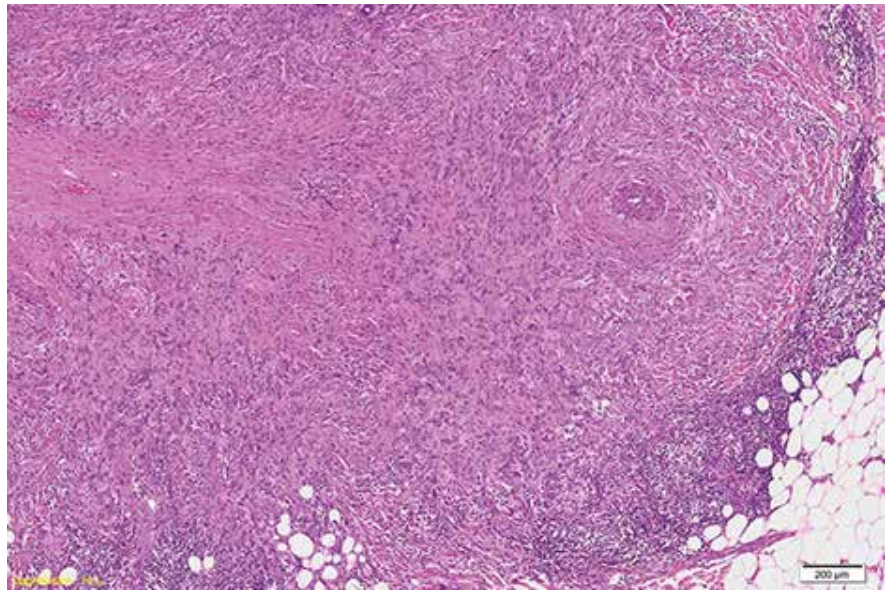

Figure 7 The myoepithelial carcinoma consisted of atypical spindle-shaped cells with infiltrative growth (Case 18)

Table 3 Immunohistochemical Results

Case	CK	EMA	ASMA	Calponin	S100	GFAP
3	+	nd	nd	nd	+	nd
9	nd	nd	f+	nd	+	nd
10	+	nd	f+	f+	+	+
11	f+	+	+	+	f+	nd
12	+	nd	+	nd	nd	-
13	-	+	-	nd	+	nd
14	+	f +	nd	f+	+	nd
15	+	nd	f+	nd	f+	nd
16	-	+	+	nd	f+	nd
17	+	nd	-	nd	+	nd
18	+	+	+	nd	-	nd
Total +						
	8/10	5/5	7/9	3/3	9/10	1/2
	80%	100%	78%	100%	100%	50%

nd, not done; f, focally

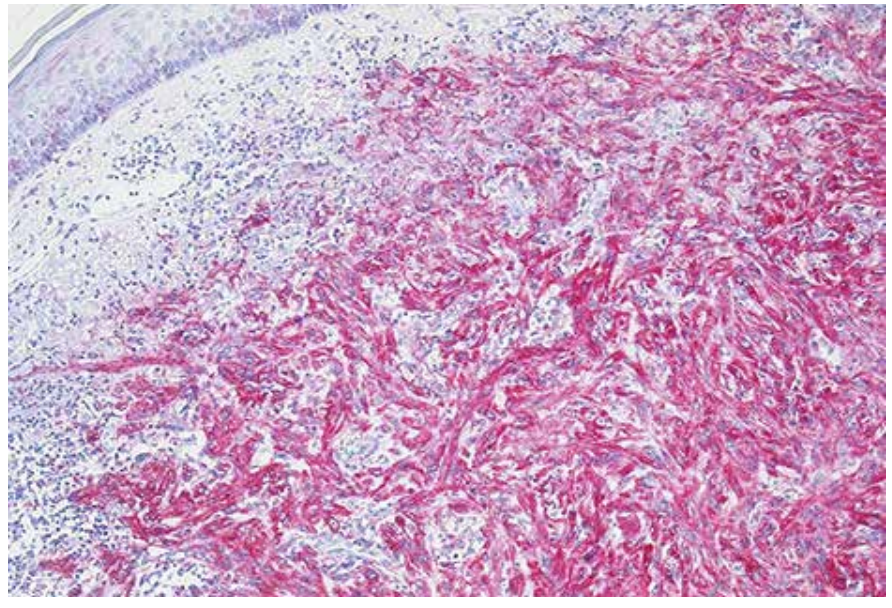

Figure 8 The atypical spindle cells expressed EMA in Case 18

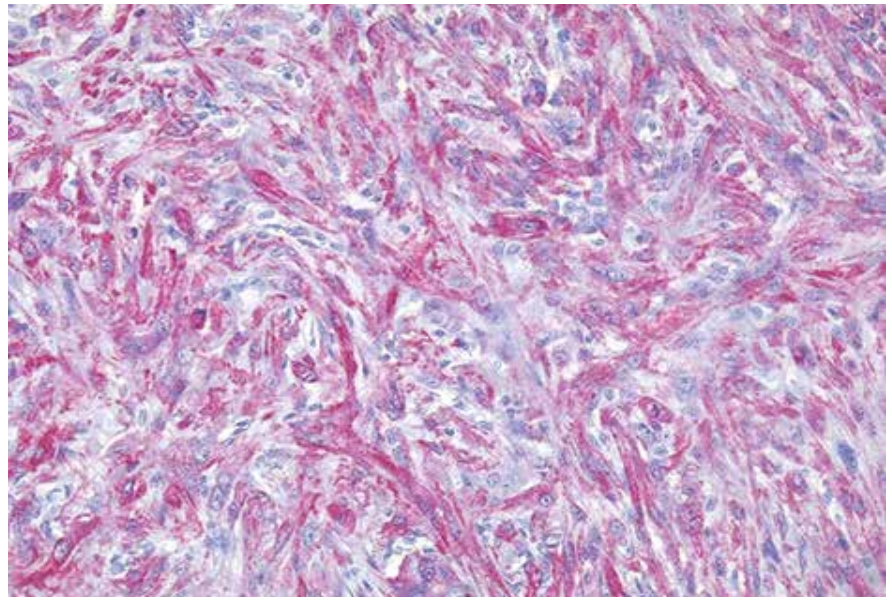

Figure 9 Expression of ASMA in the myoepithelial carcinoma

Table 4 FISH results for *EWSR1 and FUS* rearrangement

Case	*EWSR1*		*FUS*
1	+	(17/50)	nd
2	+	(19/50)	nd
3	+	(11/50)	nd
4	+	(12/50)	nd
5	-		-
6	x		-
7	-		-
8	-		-
9	-		-
10	+	(14/50)	nd
11	-	(8/50)	nd
12	-		-
13	-		-
14	-		-
15	+	(12/38)	nd
16	x		x
17	-		-
18	+	(10/50)	nd

+, break apart signal/rearrangement (in out of nuclei counted); -, no break apart signal/rearrangement; nd, not done; x, analysis failed

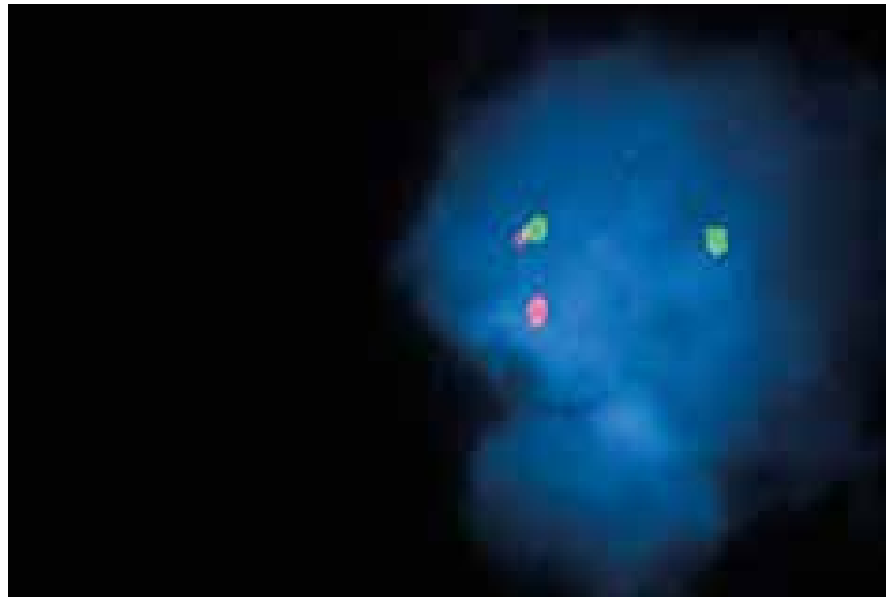

Figure 10 FISH of a myoepithelioma (Case 15) with a break apart signal for *EWSR1*

Discussion

Cutaneous myoepithelial tumors are divided into mixed tumors and myoepitheliomas depending on the occurrence of ductal structures defining the former.[2,3] They also include myoepithelial carcinomas which are defined by atypical cytological features.[3,4] In the absence of atypia, high mitotic index, local invasion and tumor necrosis should raise concern.[4,8]

Earlier studies suggested that cutaneous myoepitheliomas and their soft tissue counterparts represent points along a clinicopathologic spectrum that was apparently extended to include myoepithelial tumors of the bone and of some visceral sites (lung). [1,2,5,10] Although this entity, in the above described localizations, is well-established by now, the protean morphological and immunohistochemical range is still hampering the histopathological diagnosis, and the true frequency of these neoplasms is unclear until today.

This study also mirrors the morphological and immunohistochemical heterogeneity by the presence of a variable (immuno)phenotype in all cases. A (chondro)myxoid/hyaline matrix was almost always existent and adipocytes as a heterologous element were seen in four of our cases. All these observations are well-reported earlier.[1-4,6]

The occurrence of *EWSR1* rearrangement in mixed tumors, myoepitheliomas and rare myoepithelial carcinomas emphasizes that these lesions belong to a spectrum of cutaneous myoepithelial neoplasms.

Furthermore, *EWSR1* rearrangement found in 44% of cutaneous myoepithelial tumors in our series, provide a genetic link with their counterparts in soft tissue, bone and the lung. Most of the reported *EWSR1* rearrangements occur in soft tissue myoepithelial tumors which means that 45% of them show this abnormality.[10] This is in concert with our results in cutaneous tumors. *POUF5F1* is one of the described fusion genes in the soft tissue tumors and is associated with clear cell morphology and younger age.[10] Other known fusion partners are *PBX1* and *ZNF444*.[10-12] In 42% of the described *EWSR1* rearranged cases these fusion partners have been identified so far. For *PBX1* and *POU5F1* the same frequency was detected and a *EWS1-ZNF444* fusion was seen in only one case of the lung.[10]

When we consider the low incidence of *FUS* rearrangement in the series by Antonescu et al.[10] with one positive pulmonal myoepithelial tumor of total 66 cases, it is not surprising that we did not find a *FUS* gene rearrangement in our cases.[10]

Hidradenoma, a biphasic epithelial skin appendage tumor with occurrence of ductal/glandular and cystic structures, earlier thought to be a clear cell myoepithelioma,[13,14] possess in a subset of cases the specific translocation *CRTC1-MAML2,* which has been also described in mucoepidermoid carcinomas and Warthin's tumors of salivary glands.[15,16] One study demonstrated the occurrence of *EWSR1- POUF5F1* fusion in hidradenomas,[17] whereas Antonescu et al[10] did not find an *EWSR1* rearrangement in five eccrine hidradenomas.[10] Therefore, one could speculate whether these described lesions by Möller et al are more related to myoepithelial tumors.[17]

The much more common occurring and investigated myoepithelial tumors of the salivary glands are known for a different genetic background, which contains rearrangements of *PLAG1* and *HMGA2*.[18]

A differential diagnosis of cutaneous myoepithelial tumors in our opinion represents epithelioid sarcoma, especially the very rarely occurring myxoid variant. Their occurrence is mostly as in myoepitheliomas at the distal extremities. This myxoid variant always shows at least focally classical features of epithelioid sarcoma.[19] Although there is an immunohistochemical overlap with positivity for keratin and EMA, INI1 protein, absent in the majority of cases of epithelioid sarcoma, is a reliable marker for supporting the diagnosis.[20-22] In contrast, cutaneous myoepithelial tumors have a retained INI1 protein in all of our cases (data not shown). In this context, it should be noted that in a subset of myoepithelial carcinomas of soft tissue the INI1 protein is absent.[9] Another helpful discriminating marker is CD34 which is at least in 50% of epithelioid sarcomas positive.[20,23,24] Although genetic aberrations in *INI1,* located on 22q11, are reported in epithelioid sarcoma, *EWSR1* rearrangements are never described.[19,22,25,26]

On histomorphological grounds, extraskeletal myxoid chondrosarcomas and ossifying fibromyxoid tumors may also enter the differential diagnosis. Although extraskeletal myxoid chondrosarcomas are classically located in the deep soft tissues, ossifying fibromyxoid tumors are commonest located in the subcutis and involvement of the skin has rarely been reported. These tumors show a distinctive lobulation and in most cases a bone shell is evident.[27,28,29] Metaplastic bone formation known in soft tissue myoepithelial tumors is not described in skin lesions.[1-8] Moreover, the intratumoral cytological and architectural heterogeneity of myoepithelial tumors is not a feature of ossifying fibromyxoid tumors in which uniform round to oval cells in cords and nests set in a myxohyaline stroma.[4,6,7] Immunohistochemical stains in this setting are of limited value because of the overlap with positivity for S100, GFAP, myogenic markers and rarely also keratins.[4,6,7,27-29] Even though in a small number of cases investigated, ossifying fibromyxoid tumors show no abnormality in *EWSR1*.[10]

In summary, our study demonstrated that cutaneous myoepithelial tumors harbor *EWSR1* rearrangement in a subset of cases and therefore are genetically related to soft tissue and bone myoepithelial tumors and the visceral counterpart in the lung. Regarding the fusion genes of *EWSR1* in cutaneous tumors, further investigations are mandatory.

In addition, the relative low incidence of *EWSR1* rearrangement in myoepithelial tumors comparing with other tumors harboring translocations, alternative genetic mechanisms, more specifically, rearrangements of other genes have yet to be discovered. Apparently plays *FUS* here a tangential role.[10]

References

1. Michal M, Miettinen M. Myoepitheliomas of the skin and soft tissues. Report of 12 cases. Virchows Arch 1999;434:393-400.
2. Kutzner H, Mentzel T, Kaddu S, Soares LM, Sangueza OP, Requena L. Cutaneous myoepithelioma: an under-recognized cutaneous neoplasm composed of myoepithelial cells. Am J Surg Pathol 2001;25:348-355.
3. Mentzel T, Requena L, Kaddu S, Soares de Aleida LM, Sangueza P, Kutzner H. Cutaneous myoepithelial neoplasms: clinicopathologic and immunohistochemical study of 20 cases suggesting a continuous spectrum ranging from benign mixed tumor of the skin to cutaneous myoepithelioma and myoepithelial carcinoma. J Cutan Pathol 2003;30:294-302.
4. Hornick JL, Fletcher CDM. Cutaneous myoepithelioma: A clinicopathologic and immunohistochemical study of 14 cases. Hum Pathol 2004;35:14-24.
5. Kilpatrick SE, Hitchcock MG, Kraus MD, Calonje E, Fletcher CDM. Mixed tumors and myoepitheliomas of soft tissue: A clinicopathologic study of 19 cases with a unifying concept. Am J Surg Pathol 1997;21:13-22.
6. Gleason BC, Hornick JL. Myoepithelial tumours of skin and soft tissue: an update. Diagn Histopathol 2008;14:552-562.
7. Hornick JL, Fletcher CDM. Myoepithelial tumors of soft tissue. A clinicopathologic and immunohistochemical study of 101 cases with evaluation of prognostic parameters. Am J Surg Pathol 2003;27:1183-1196.
8. Bates AW, Baithun SI. Atypical mixed tumor of the skin: Histologic, immunohistochemical, and ultrastructural features in three cases and a review of the criteria for malignancy. Am J Dermatopathol 1998;20:35-40.
9. Gleason BC, Fletcher CDM. Myoepithelial carcinoma of soft tissue in children: An aggressive neoplasm analysed in a series of 29 cases. Am J Surg Pathol 2007;31:1813-1824.
10. Antonescu CR, Zhang L, Chang NE, Pawel BR, Travis W, Katabi N, Edelman M, Rosenberg AE, Nielsen GP, Dal Cin P, Fletcher CDM. *EWSR1-POU5F1* fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone and visceral lesions showing common involvement of EWSR1 gene rearrangement. Genes Chromosomes Cancer 2010;49:1114-1124.
11. Brandal P, Panagopoulos I, Bjerkehagen B, Gorunova L, Skjeldal S, Micci F, Heim S. Detection of a t(1;22)(q23;q12) translocation leading to an *EWSR1-PBX1* fusion gene in a myoepithelioma. Genes Chromosomes Cancer 2008;47:558-564.
12. Brandal P, Panagopoulos I, Bjerkehagen B, Heim S. t(19;22)(q13;q12) Translocation leading to the novel fusion gene *EWSR1-ZNF444* in soft tissue myoepithelial carcinoma. Genes Chromosomes Cancer 2009;48:1051-1056.
13. Lever WF. Myoepithelial sweat gland tumor, myoepithelioma; report of three cases with a review of the literature. Arch Derm Syphilol 1948;57:332-347.
14. Requena L, Kiryu H, Ackerman AB. Apocrine hidradenoma. Neoplasms with apocrine differentiation. Lippincott-Raven: Philadelphia, PA; 1998, pp243.
15. Winnes M, Molne L, Suurkula M, Andren Y, Persson F, Enlund F, Stenman G. Frequent fusion of the CRTC1 and MAML2 genes in clear cell variants of cutaneous hidradenomas. Genes Chromosomes Cancer 2007;46:559-563.
16. O'Neill ID. t(11;19) translocation and *CRTC1-MAML2* fusion oncogene in mucoepidermoid carcinoma. Oral Oncol 2009;45:2-9.
17. Möller E, Stenman G, Mandahl N, Hamberg H, Mölne L, van den Oord JJ, Brosjö O, Mertens F, Panagopoulos I. POU5F1, encoding a key regulator of stem cell pluripotency, is fused to EWSR1 in hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands. J Pathol 2008;215:78-86.
18. Stenman G. Fusion oncogenes and tumor type specifity – insights from salivary gland tumors. Semin Cancer Biol 2005;15:224-235.
19. Flucke U, Hulsebos TJM, van Krieken JHJM, Mentzel T. Myxoid epithelioid sarcoma: a diagnostic challenge. A report on six cases. Histopathology 2010;57:753-759.

20. Chbani L, Guillou L, Terrier P, Decouvelaere AV, Grégoire F, Terrier-Lacombe MJ, Ranchère D, Robin YM, Collin F, Fréneaux P, Coindre JM . Epithelioid sarcoma. A clinicopathologic and immunohistochemical analysis of 106 cases from the French Sarcoma Group. Am J Clin Pathol 2009;13:222-227.
21. Hornick JL, Dal Cin P, Fletcher CDM. Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. Am J Surg Pathol 2009;33:542-550.
22. Kohashi K, Izumi T, Oda Y, Yamamoto H, Tamiya S, Taguchi T, Iwamoto Y, Hasegawa T, Tsuneyoshi M. Infrequent SMARCB1/INI1 gene alteration in epithelioid sarcoma: a useful tool in distinguishing epithelioid sarcoma from malignant rhabdoid tumor. Hum Pathol 2009;40:349-355.
23. Guillou L, Wadden C, Coindre JM, Krausz T, Fletcher CDM. Proximal-type epithelioid sarcoma, a distinctive aggressive neoplasm showing rhabdoid features: clinicopathologic, immunohistochemical, and ultrastructural study of a series. Am J Surg Pathol 1997;21: 130-146.
24. Miettinen M, Fanburg-Smith JC, Virolainen M, Shmookler BM, Fetch JF. Epithelioid sarcoma: an immunohistochemical analysis of 112 classical and variant cases and a discussion of the differential diagnosis. Hum Pathol 1999;30:934-942.
25. Modena P, Lualdi E, Facchinetti F, Galli L, Teixeira MR, Pilotti S, Sozzi G. SMARCB1/INI1 tumor suppressor gene is frequently inactivated in epithelioid sarcoma. Cancer Res 2005;65:4012-4019.
26. Gasparini, P, Facchinetti F, Boeri M, Lorenzetto E, Livio A, Gronchi A, Ferrari A, Massimino M, Spreafico F, Giangaspero F, Forni M, Maestro R, Alaggio R, Pilotti S, Collini P, Modena P, Sozzi G. Prognostic determinants in epithelioid sarcoma. Eur J Cancer 2011;47:287-295.
27. Fletcher CDM, Unni K, Mertens F, eds. World Health Organization Classification of Tumours. Pathology and Genetics of Tumors of Soft tissue and Bone. IARC press: Lyon, France; 2002. 196 pp, 213pp.
28. Folpe AL, Weiss SW. Ossifying fibromyxoid tumor of soft parts. A clinicopathologic study of 70 cases with emphasison atypical and malignant variants. Am J Surg Pathol 2003;27:421-431.
29. Miettinen M, Finnell V, Fetsch JF. Ossifying fibromyxoid tumor of soft parts – A clinicopathologic and immunohistochemical study of 104 cases with long-term follow-up and a critical review of the literature. Am J Surg Pathol 2008;32:996-1005.

Chapter 3

EWSR1-ATF1 chimeric transcript in a myoepithelial tumor of soft tissue: a case report

Hum Pathol 2012 May;43(5):764-8.

Abstract

Soft tissue myoepithelial tumors, a recently defined entity, include benign and malignant lesions showing a considerable morphological and immunohistochemical heterogeneity.

EWSR1 rearrangements are well recognized in this tumor type, and some of the partner genes have been identified. Herein we describe a soft tissue myoepithelioma arising in the pelvis with an *EWSR1-ATF1* fusion, therefore extending the spectrum of partner genes of *EWSR1*.

In addition, this case indicates that there are overlapping genetic features of myoepithelial tumors, clear cell sarcoma, angiomatoid fibrous histiocytoma, and hyalinising clear-cell carcinoma of salivary gland.

Introduction

Soft tissue myoepithelial tumors, a recently defined entity, include benign and malignant lesions characterized by a broad morphological and immunohistochemical spectrum [1].

The first large molecular study demonstrated *EWSR1* rearrangements in approximately 45% of soft tissue myoepithelial tumors with identified fusion genes such as *POU5F1*, *PBX1* and *ZNF444* in 42% of them.[2]
We describe herein a soft tissue myoepithelioma arising in the pelvis with an *EWSR1-ATF1* fusion, therefore extending the spectrum of partner genes of *EWSR1* in this tumor type.

Clinical History

A 57-year-old male patient had a 14 cm large tumor in his pelvis with erosion of the sacrum and displacement of urinary bladder and rectum.

A transabdominal biopsy was taken. The tumor was considered as irresectable, and the patient received radiotherapy without obvious signs of regression. Three years later, he experienced local tumor progression with sacral nerve root compression and renal insufficiency due to hydronephrosis. Death from these local problems ensued 43 months after the initial diagnosis. Metastases were not detected.

Material and methods

The tumor tissue from the biopsy was partly snap frozen and fixed in 4% buffered formalin, routinely processed, and embedded in paraffin; 2-to 4-µm thick sections were stained with hematoxylin and eosin and immunohistochemically by the labelled Streptavidin Biotin technique using commercially available antibodies listed in Table 1. Appropriate positive and negative controls were used throughout.

RNA extracts from fresh frozen tissue were subjected to reverse transcription-polymerase chain reaction (RT-PCR) and assessed for different gene fusions that were established in 2001 for sarcomas, as Ewing sarcoma (*EWSR1-FLI1/ERG*), synovial sarcoma (*SS18-SSX1/2*), extraskeletal myxoid chondrosarcoma (*EWSR1-NR4A3*), myxoid liposarcoma (*FUS-DDIT3*) and clear cell sarcoma (*EWSR1-ATF1*) because the histological and immunohistochemical features did not fit with any of the widely accepted entities at that time. The diagnosis of a clear cell sarcoma was made owing to the fusion product of *EWSR1-ATF1.* When we reevaluated this case recently, the diagnosis was changed into myoepithelioma owing to morphology and immunohisto-

chemisty. Moreover, we repeated RT-PCR for *EWSR1-ATF1* and additionally applied *EWSR1*-fluorescence in situ hybridization *(*FISH) analysis as described below:

Translocation-specific PCR

cDNA synthesis was performed in a 24-ul reaction containing 1 ug of RNA, 1 ug of random hexamers (Promega, Madison, WI, USA) and 20 nmol dNTPs (Invitrogen, Carlsbad, CA, USA) and heated at 65 °C for 5 min. Next 2 ul of RNasin (Promega), 8 ul of 5x first strand buffer (Invitrogen), 4 ul of 0.1 M DTT (Invitrogen) and 2 ul of Superscript II (Invitrogen) were added and the sample was heated accordingly: 10 minutes at 20 °C, 60 minutes at 42 °C and 3 minutes at 95 °C. cDNA was stored at -20 °C.

Potential translocation-specific *EWSR1-ATF1* fusion products were detected using primers targeting *EWSR1* (exon 7: TCCTACAGCCAAGCTCCAAGTC and exon 8: GATTTGATCGTGGAGGCATGAG, RefSeq: NM_005243.1) and *ATF1* (exon 5: GTACTCCATCTGTGCCTGG, RefSeq: NM_005171.2).

DNA amplification was performed in duplicate in a PTC 200 Thermal Cycler (MJ Research, Waltham, MA, USA). The PCR was started with 10 minutes at 95 °C and followed with 38 cycles of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds and extension at 72°C for 60 seconds, followed by a final extension at 72°C for 7 minutes and cooling down for 5 minutes at 20°C. PCR products were analyzed by 2% agarose gel electrophoresis.

Fluorescent in situ hybridization (FISH) analysis

For the detection of an *EWSR1* gene rearrangement (22q12) a direct fluorescein isothiocyanate/rhodamine-labeled break apart probe (Abbott, Bergisch Gladbach, Germany) was used.

FISH was performed on 3 µM sections of formalin-fixed, paraffin-embedded tissue after baking at 65°C for 16 hours, deparaffinization with xylene and rehydration with ethanol. All tissue sections were pretreated with a 30% solution of Oncor pretreatment powder in 2xSSC and digested for 10 minutes with proteinase K following the instructions of the suppliers (Q-Biogene, Heidelberg, Germany). After a second rehydration step, the probes were applied to the sections and the covered slides were sealed with rubber cement, heat-denatured, and hybridised at 37°C for 16 hours. All sections were counterstained with DAPI II in mounting medium (125 ng/ml, Abbott, Bergisch Gladbach, Germany) and visualized under a Zeiss Axioplan microscope using a HBO100 lamp and the appropriate filters for the 3 fluorescent dyes. A negative control was used.

Table 1 Details of used immunohistochemical antibodies

Antibody	Clone	Dilution	Source
ASMA	1A4	1:500	DAKO, Glostrup, Denmark
EMA	Mc5	1:400	BioGenex, San Ramon, USA
Pan-cytokeratin	MNF116	1:500	DAKO, Glostrup, Denmark
Pan-cytokeratin	AE1/3	1:50	DAKO, Glostrup, Denmark
CK8/18	CAM 5.2	1:50	Becton Dickinson, San Jose, USA
S-100	polyclonal	1:2000	DAKO, Glostrup, Denmark
Calponin	CALP	1:400	DAKO, Glostrup, Denmark
HMB-45	HMB-45	1:300	DAKO, Glostrup, Denmark
Melan A	Clone A103	1:50	DAKO, Glostrup, Denmark
GFAP	GA-5	1:200	BioGenex, San Ramon, USA
p 63	4A4	1:400	DAKO, Glostrup, Denmark

Results

Histologically, the tumor showed a trabecular pattern of uniform-appearing epithelioid, ovoid or focally somewhat more spindled cells with mainly round nuclei without prominent nucleoli. The limited amount of cytoplasm was clear to eosinophilic. Cytological atypia was absent and mitotic figures were scarce. There was an obvious chondromyxoid background (Figure 1).

The immunhistochemical features consisted of positivity for S-100 (Figure 2) and focally for EMA (Figure 3), whereas Pan-CK (MNF116, AE1/3), CAM5.2, α-smooth muscle actin, p63, glial fibrillary acidic protein, HMB-45, Melan-A, and calponin were negative.

The *EWSR1-ATF1* fusion detected by using RT-PCR was due to an in-frame fusion of exon 8 of *EWS* with exon 4 of the *ATF1* gene (Figure 4).

In 2 other soft tissue myoepithelial tumors tested, we did not find an EWSR1/ATF1 chimeric transcript (data not shown).

By FISH analysis, the tumor exhibited an *EWSR1* rearrangement. A break-apart signal was seen in 23 out of 50 counted nuclei (Figure 5).

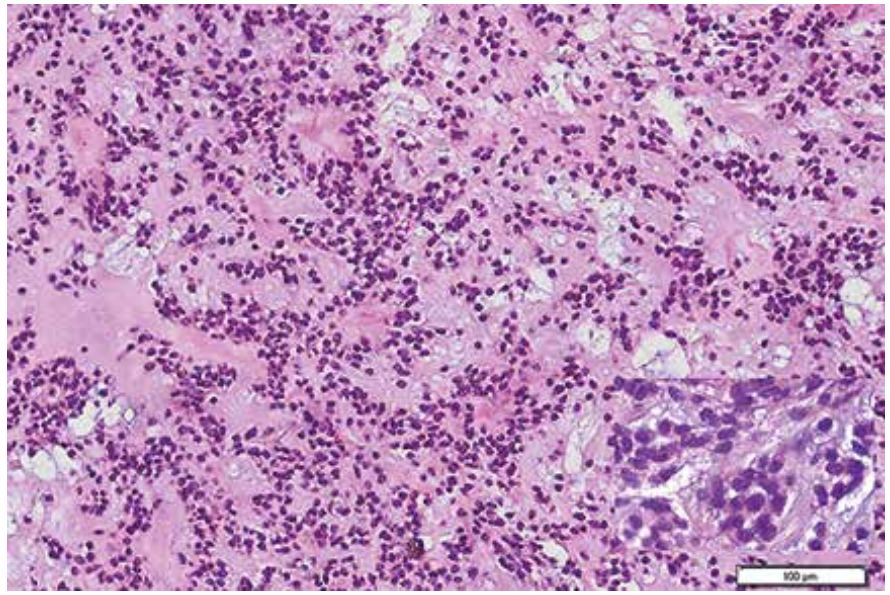

Figure 1 Trabecular and reticular arrangement of epithelioid cells set in a chondromyxoid stroma

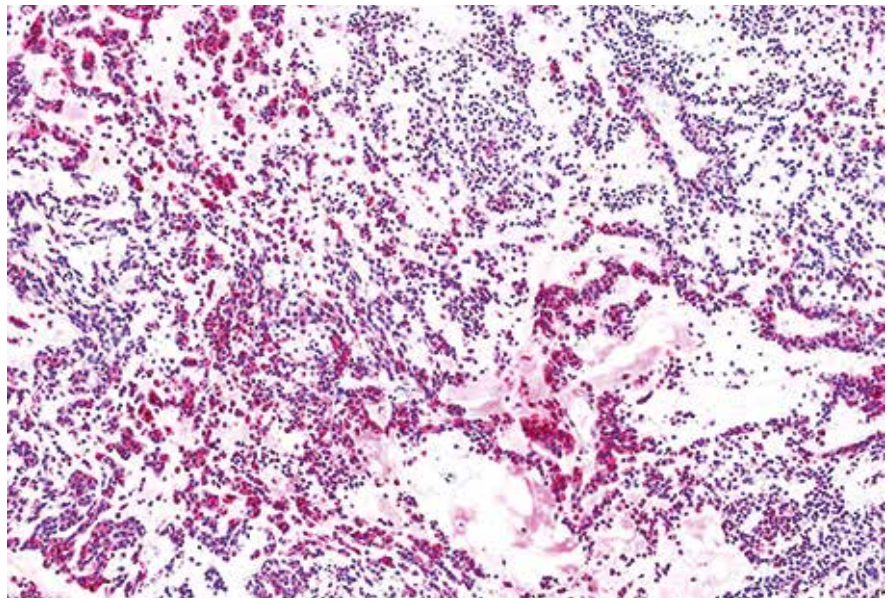

Figure 2 The tumor showed expression of S-100

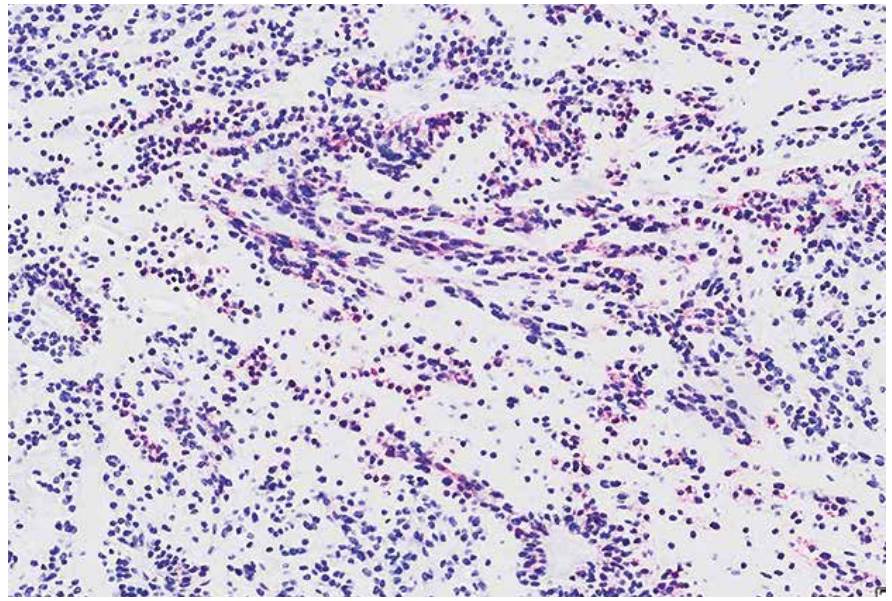

Figure 3 Immunostaining for EMA was focal

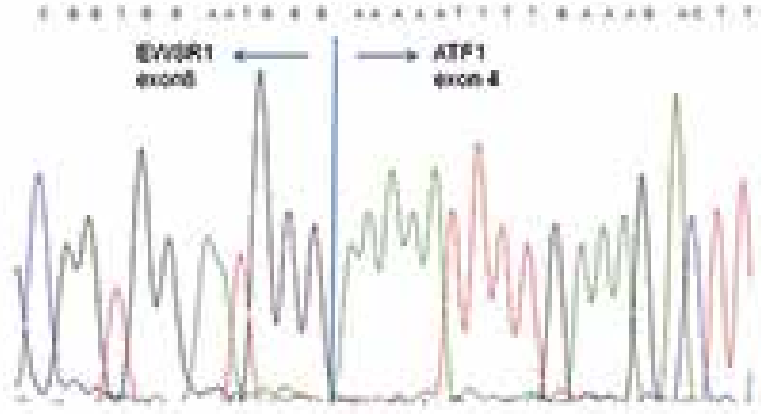

Figure 4 Sequence of RT-PCR product showing a fusion of *EWSR1* exon 8 to *ATF1* exon 4 in the chimeric transcript

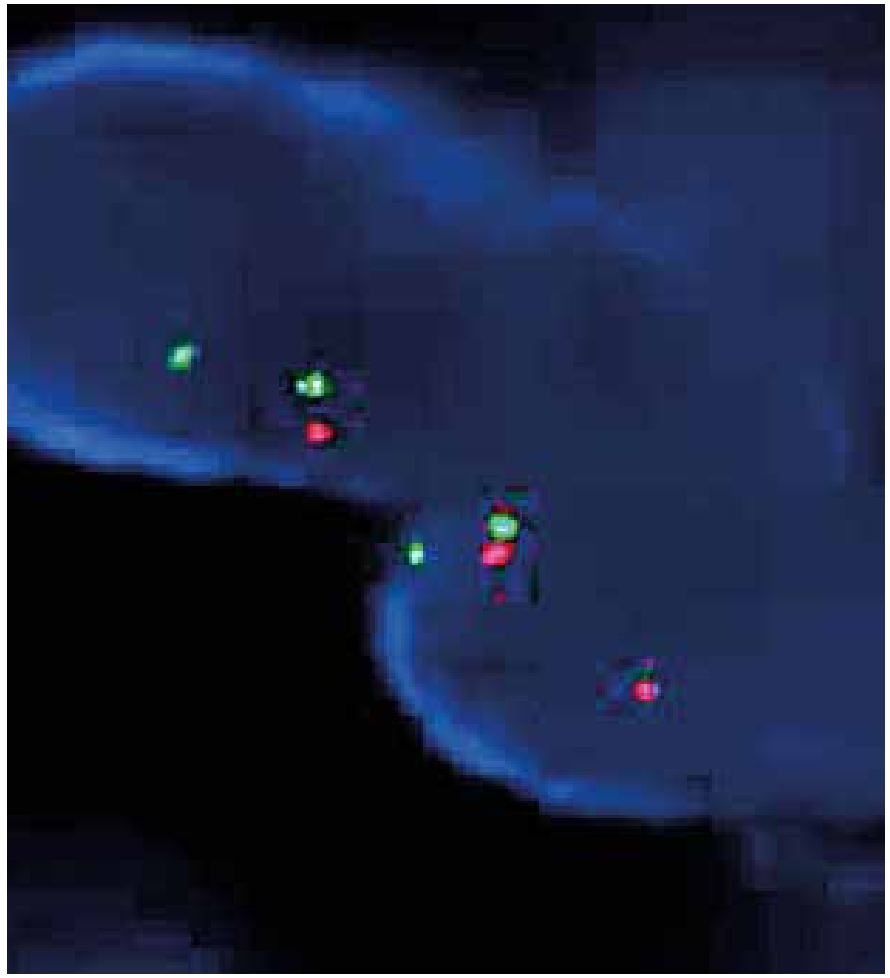

Figure 5 *EWSR1* gene rearrangement by FISH (break-apart signal)

Discussion

Stout and Gorman described the first case of a soft tissue myoepithelial tumor in 1959, and Kilpatrick et al.[4] extended the concept of this tumor type by including metastatic cases. When Hornick and Fletcher[5] reported on the largest series to date, they proposed moderate to severe atypia as criterion for malignancy. A considerable heterogeneity in architecture, cytology, stroma reaction and immunohistochemistry owing to the flexibility of myoepithelial cells is a hallmark of this entity.[1,5,6]

Recently, *EWSR1* gene rearrangements were detected in both benign and malignant myoepithelial tumors of soft tissue, bone, skin, and the lung. The identified partner genes, found in approximately 20% of all cases, are *POU5F1*, *PBX1* and *ZNF444*. Most of these fusions correlated with deep soft tissue localization. Furthermore, distinctive clear cell morphology and young patient age were strongly associated with *EWSR1-POU5F1.*[2]

Until now*, EWSR1-ATF1* has never been described in myoepithelial tumors, but it is well known in clear cell sarcoma and angiomatoid fibrous histiocytoma.[6] In a recently published study, Antonescu et al.[7] demonstrated the occurrence of this gene fusion also in hyalinising clear-cell carcinoma of salivary gland and reported novel break points (*EWSR1* exon 11; *ATF1* exon 3). Interestingly, these tumors show superficially resemblance with some of the myoepithelial soft tissue tumors, but lack true myoepithelial features by immunohistochemistry.[7] On the contrary, myoepithelial differentiation was supposed by others based also on immunohistochemical data.[8]

Clear cell sarcomas, mostly present on the distal extremities in young adults, have also been reported at unusual sites, such as pelvis, kidney, gastrointestinal tract, skin, breast and mediastinum.[6,9-16] Although, classical histological features as first described by Enzinger[9] are present in the majority of cases, morphological variations are also recorded. The latter include an alveolar and seminomalike pattern, rhabdoid cellular features, polymorphic cells and small intercellular pools of mucoid material.[9,12,13,17]

In contrast, our case consisted of uniform-appearing epithelioid cells arranged in a trabecular pattern. Prominent nucleoli, a cytological hallmark of clear cell sarcoma, were not discernible. As is typical for myoepithelial tumors, a prominent chondromyxoid matrix was present. On the other hand, a nested and short fascicular growth pattern accompanied by a delicate fibrous network in clear cell sarcoma and wreath-like multinucleated giant cells were absent.[6]

EMA and S100, positive in the here described case, show variable coexpression with myogenic markers (calponin, α-smooth muscle actin, desmin), glial fibrillary acidic protein, and keratins in myoepithelial tumors.[1,4-6]

However, a recent study described EMA positivity in one third of the cases of clear cell sarcoma but the overwhelming majority of cases expressed S100 in combination with HMB45 and/or Melan A and/or MITF.[13] Traces of keratin expression in clear cell sarcomas were rarely described.[14]

Angiomatoid fibrous histiocytoma, with its distinct morphology, is easily distinguishable from myoepithelial tumors. This mostly superficially localized circumscribed lesion is defined by sheets of bland spindle to ovoid cells, peripheral lymphocytic infiltrate and blood-filled cystic cavities and behaves mainly in a benign fashion. Expression of desmin and muscle actin in a subset of cases led to the hypothesis of a myoid nature. EMA can also be positive and is therefore not discriminating in this setting.[6]

In summary, our case represented a myoepithelial neoplasm of soft tissue with an *EWSR1-ATF1* chimeric transcript. A malignant nature of this tumor was not obvious, because of its bland cytomorphology. However, the unfavourable localization of a large tumor within the pelvis made surgery impossible and a locally aggressive behaviour despite radiotherapy resulted in death of the patient 3.5 years after the initial diagnosis.

Additionally, this case indicates that there are overlapping genetic features of myoepithelial tumors, clear cell sarcoma, angiomatoid fibrous histiocytoma and hyalinising clear-cell carcinoma of salivary gland. The differences in morphology and immunohistochemistry between the mentioned soft tissue lesions allow their distinction, which, naturally, is mandatory for patient management.

References

1. Gleason BC, Hornick JL. Myoepithelial tumours of skin and soft tissue: an update. Diagn Histopathol 2008;14:552-562.
2. Antonescu CR, Zhang L, Chang NE, et al. *EWSR1-POU5F1* fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone and visceral lesions showing common involvement of *EWSR1* gene rearrangement. Genes Chromosomes Cancer 2010;49:1114-1124.
3. Stout AP, Gorman JG. Mixed tumors of the skin of the salivary gland type. Cancer 1959;12:537-543.
4. Kilpatrick SE, Hitchcock MG, Kraus MD, Calonje E, Fletcher CDM. Mixed tumors and myoepitheliomas of soft tissue: A clinicopathologic study of 19 cases with a unifying concept. Am J Surg Pathol 1997;21:13-22.
5. Hornick JL, Fletcher CDM. Myoepithelial tumors of soft tissue. A clinicopathologic and immunohistochemical study of 101 cases with evaluation of prognostic parameters. Am J Surg Pathol 2003;27:1183-1196.
6. Fletcher CDM, Unni K, Mertens F, eds. World Health Organization Classification of Tumours. Pathology and Genetics of Tumors of Soft tissue and Bone. IARC press: Lyon, France; 2002. 194pp, 198pp, 211pp.
7. Antonescu CR, Katabi N, Zhang L, et al. EWSR1-ATF1 fusion is a novel and consistent finding in hyalinizing clear-cell carcinoma of salivary gland. Genes Chromosomes Cancer 2011;50:559-570.
8. Ellis GL, Auclair PL, eds. AFIP Atlas of Tumor Pathology Series 4. Tumors of the salivary glands. ARP press: Silver Spring, Maryland; 2008. 301pp.
9. Enzinger F. Clear-cell sarcoma of tendons and aponeuroses. An analysis of 21 cases. Cancer 1965;18:1163-1174.
10. Rubin BP, Fletcher JA, Renshaw AA. Clear cell sarcoma of soft parts: report of a case in the kidney with cytogenetic confirmation. Am J Surg Pathol 1999;23:589-594.
11. Zambrano E, Reyes-Mugica M, Franchi A, Rosai J. An osteoclast-rich tumor of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: reports of 6 cases of a GIST simulator. Int J Surg Pathol 2003;11:75-81.
12. Antonescu CR, Nafa K, Segal NH, Dal Cin P, Ladanyi M. *EWS-CREB1*: a recurrent variant fusion in clear cell sarcoma – association with gastrointestinal location and absence of melanocytic differentiation. Clin Cancer Res 2006;12:5356-5362.
13. Hisaoka M, Ishida T, Kuo TT, et al. Clear cell sarcoma of soft tissue. A clinicopathologic, immunohistochemical, and molecular analysis of 33 cases. Am J Surg Pathol 2008;32:452-460.
14. Tirabosco R, Lang-Lazdunski L, Diss TC, et al. Clear cell sarcoma of the mediastinum. Ann Diagn Pathol 2009;13:197-200.
15. Wang WL, Mayordomo E, Zhang W, et al. Detection and characterization of *EWSR1/ATF1* and *EWSR1/CREB1* chimeric transcrips in clear cell sarcoma (melanoma of soft parts). Mod Pathol 2009;22:1201-1209.
16. Hantschke M, Mentzel T, Rütten A, et al. Cutaneous clear cell sarcoma: a clinicopathologic, immunohistocchemical, and molecular analysis of 12 cases emphasizing its distinction from dermal melanoma. Am J Surg Pathol 2010;34:216-222.
17. Sara AS, Evans HL, Benjamin RS. Malignant melanoma of soft parts (clear cell sarcoma). A study of 17 cases, with emphasis on prognostic factors. Cancer 1990;65:367-374.

Chapter 4

NR4A3 rearrangement reliably distinguishes between the clinicopathologically overlapping entities myoepithelial carcinoma of soft tissue and cellular extraskeletal myxoid chondrosarcoma

Virchows Arch 2012 Jun;460(6):621-8.

Abstract

Myoepithelial carcinoma of soft tissue (MEC) and cellular extraskeletal myxoid chondrosarcoma (cEMC) share striking similarities. In this paper, we compare ten MECs with 5 cEMCs.

MEC patients had an equal gender distribution. The age range was 15-76 years (mean, 42 years). Tumors were located on extremities, pelvic girdle, vulva, and neck. Follow-up, available for 9 patients, ranged from 4 to 85 months (mean, 35 months). Five patients were alive without evidence of disease, two were alive with disease and two died 8 months after the initial diagnosis. cEMCs were from three males and two females with an age range of 37-82 years (mean, 57 years); they presented in extremities, shoulder and paravertebral/cervical. Follow-up, available for four patients, ranged from 6 to 220 months (mean, 61 months). All patients were alive, two with recurrences and/or metastases and two without evidence of disease. Morphologically, the distinction between these two entities was difficult since all cases exhibited features typically seen in myoepithelial tumors. Immunohistochemically, MECs expressed pan-keratin (80%), epithelial membrane antigen (57%), S100 (50%), alpha-smooth muscle actin (ASMA; 75%), calponin (67%) and p63 (25%). S100 and EMA were expressed in 40% of cEMC cases respectively with additional immunoreactivity for p63, ASMA and glial fibrillary acidic protein in one case. Pan-keratin was negative in all neoplasms. *NR4A3* rearrangement was present in four of four cEMCs and in none of the MECs. In contrast, three of nine (33%) MECs and four of five (80%) cEMCs showed an *EWSR1* rearrangement. In summary, MECs and cEMCs share clinical, morphological, immunohistochemical and genetic characteristics. The pathognomic rearrangement of *NR4A3* is a useful diagnostic feature in identifying cEMCs.

Introduction

Myoepithelial tumors of the soft tissue were initially characterized in a series by Kilpatrick et al.[1] Later on, criteria for malignancy were established and the clinico-pathological features were expanded.[2,3] Histologically, these tumors show the same broad variation in morphology as seen in their salivary gland counterparts.[1-4]

Extraskeletal myxoid chondrosarcomas (EMCs) reveal a cord- or lace-like arrangement of small round to spindle-shaped cells with distinct eosinophilic cytoplasm distributed in a prominent myxoid stroma. In cellular lesions (cEMCs), amounting to one third of the cases, there is a greater morphological diversity.[5-11]

Although the age range is broad for both entities, the peak incidence for EMC is the sixth and for myoepithelial tumors the fourth decade.[2,4,12] Furthermore, a significant subset of malignant myoepithelial tumors or myoepithelial carcinomas (MECs) occurs in children in contrast to EMCs, which show a very low incidence in this cohort.[3,5,8,12,13] Both, myoepithelial tumors and EMCs arise predominantly in the proximal lower extremity but EMCs are more often located in the deep soft tissue.[2,4,12]

Whereas EMCs have a protracted clinical course with a 10-year survival rate up to 88%, [5,8,14-16] MECs typically demonstrate aggressive behavior, particularly in the pediatric population.[2,3] Whether or not also cEMCs have a worse prognosis remains controversial.[5-10]

Histologically, cEMC can be morphologically similar to myoepithelial tumors but the latter have, in most of the cases, a broader immunohistochemical profile.[2-4,12] Gene fusions involving *NR4A3 (nuclear receptor subfamily 4, group A ,member 3)* located at 9q22 are characteristic for EMC and have never been described in myoepithelial tumors. In contrast, both tumor types harbor *EWSR1 (Ewing sarcoma breakpoint region 1;* 22q12.2*)* rearrangement.[3,12,17-25]

In this paper, we report herein on the morphological, immunohistochemical and molecular overlap of MEC and cEMC.

Material and Methods

We searched the surgical pathology and referral files of the authors for the diagnoses myoepithelial tumors of soft tissue and EMC. Slides were reviewed and the diagnoses were based on criteria according to the recent WHO classification.[26] Myoepithelial tumors with moderate to severe nuclear atypia (vesicular or coarse chromatin, prominent, often large nucleoli, or nuclear pleomorphism) and EMCs with cellular features were included in our study. Clinical details and follow-up were obtained from the referring pathologists (see acknowledgement). Cases 8 and 10 were published earlier.[3,27,28]

Table 1 Details of used immunohistochemical antibodies

Antibody	Clone	Dilution	Source
ASMA	1A4	1:500	DAKO, Glostrup, Denmark
EMA	Mc5	1:400	BioGenex, San Ramon, USA
CD34	HPCA-1	1:100	BD Biosciences, San Jose, USA
Pan-cytokeratin	MNF116	1:500	DAKO, Glostrup, Denmark
Pan-cytokeratin	AE1/3	1:50	DAKO, Glostrup, Denmark
S-100 protein	polyclonal	1:2000	DAKO, Glostrup, Denmark
P63	4A4	1:5000	Thermo Fisher Scientific, USA
GFAP	GA-5	1:200	DCS, Hamburg, Germany
Calponin	CALP	1:400	DAKO, Glostrup, Denmark

In all cases the tissue was fixed in 4% buffered formalin, routinely processed and embedded in paraffin; 2-4 µm thick sections were stained with hematoxylin and eosin. Immunohistochemistry methods consisted of the labelled Streptavidin Biotin technique using commercially available antibodies as listed in Table 1. Appropriate positive and negative controls were used throughout.

Fluorescence in situ hybridization analysis

Fluorescence in situ hybridization (FISH) was performed as described earlier. For *EWSR1* a directly FITC/Rhodamine-labeled break apart-probe (Abbott, Bergisch Gladbach, Germany) was used.[29] FISH probes to detect a *NR4A3* rearrangement (break apart-probe) were generated in-house. BAC clones RP11-624K13 (centromeric), RP11-412F16 (centromeric), RP11-121L12 (telomeric) and RP11-467B11 (telomeric) were obtained from the BACPAC Resources Center (Oakland, CA). Clones were either labeled with biotin or digoxigenin (DIG) using a nick translation kit, according to manufacturer's instructions (Roche, Basel, Switzerland). Copy numbers of chromosome 9 were assessed using a centromere probe (CEP9). A negative control has been used for each tumor. A case was considered having a break when at least 10 of 50 counted tumor cells (20%) showed separation of a red and green signal.

Reverse transcription polymerase chain reaction (RT-PCR)

RNA was isolated from formalin-fixed, paraffin-embedded material by proteinase K digestion, followed by phenol/chloroform extraction and *n*-propanol precipitation. cDNA synthesis was performed in a 24-µl reaction containing 1 µg of RNA, 1 µg of random hexamers (Promega) and 20 nmol dNTPs (Invitrogen) and heated at 65 °C for 5 min. Next, 2 µl of RNasin (Promega), 8 µl of x 5 first strand buffer (Invitrogen), 4 µl

of 0.1 M DTT (Invitrogen) and 2 µl of Superscript II (invitrogen) were added and the sample was heated accordingly: 20 °C for 10 min, 42 °C for 60 min and 95 °C for 3 min. For EMC, most potential translocation-specific *EWSR1-NR4A3* and *TAF15-NR4A3* fusion products were detected using primers targeting *EWSR1* (exon 7: TCCTACAGC-CAAGCTCCAAGTC and exon 11: GACTCTAGATGATCTGGCAGAC, RefSeq: NM_005243.3), *TAF15 (TAF15 RNA polymerase II)* (exon 6: AGCAGTCAAATTATGATCAGCAGC, RefSeq: NM_003487.2) and *NR4A3* (exon 3: CCTGGAGGGGAAGGGCTATATTGGG, RefSeq: NM_006981.3).

Results

Clinical Findings

Clinical details are summarized in Table 2. The MEC cohort consisted of five males and five females with an age range of 15-76 years (mean, 42 years). The tumors were located on the extremities (n=4) with one case each on the thigh, calf, forearm and hand. Other sites were vulva (n=2), gluteal (n=1), sacral (n=1), and the neck region (n=1). In one case, the exact anatomic site was not known. Seven MECs were situated in the deep soft tissue and two subcutaneous. One superficially located tumor of the vulva showed exophytic growth. All patients underwent surgical treatment. Complete resection was reached in seven cases and marginal excision in one case. Tumor-positive margins were reported in two cases. Follow-up, available for nine patients, ranged from 4 to 85 months (mean, 35 months). Five patients were alive without evidence of disease at 12, 34, 39, 60 and 70 months, respectively. Two patients were alive with disease 4 and 85 months after the initial diagnosis, the latter with a second local recurrence. Two patients died of tumor 8 months after the initial diagnosis. Case 10 was the first local recurrence on the hand with secondary bone involvement. Three patients presented with lung metastases. Involvement of regional lymph nodes was additionally observed in two of them, and one patient had bone metastases.

The five cases of EMC were from three males and two females with an age range 37-82 years (mean, 57 years). Two lesions were located in the deep soft tissue of the thigh and one in the subcutis and soft tissue of the shoulder. One tumor each arose at the ankle and paravertebral/cervical region. All patients but one (n=4) underwent resection. Tumor free resection margins were reported in three cases (wide margins in two cases and marginal resection in one case). In Case 14, the resection status was not known. Follow-up, available for four patients, ranged from 6 to 220 months (mean, 61 months). No patient died of disease so far. Case 15 was the 4th recurrence of a tumor with primarily classical morphological features. This patient had also pulmonary spread. Another patient was known with regional lymph node involvement (Case 13).

Table 2 Clinical data

Case Sex/Age	Localization	Size (cm)	Treatment	Follow-up (mos)	Rec, Met	
MEC						
1	f/36y	na/sc	4.5	R0	NA	
2	f/21y	thigh/deep	5.0	R0	70, NER	
3	f/53y	vulva/exophytic	3.5	R0	60, NER	
4	f/75y	vulva	3.5	R0	34, NER	
5	m/36y	forearm/deep	10	R0	8, DOD	lung
6	f/15y	neck/deep	4	R2	85, AWD	2 rec
7	m/15	sacral/deep	5	R0	39, NER	
8	m/17y	calf/deep	12	R0, perf,amp	8, DOD	bone, lung, LN
9	m/71y	buttock/sc	15	R1	4, AWD	lung, LN
10	m/76y	hand/deep, bone invasion	4.5	RM	12, NER	1 rec
EMC						
11	m/61y	thigh/deep	8.1	R0	12, NER	
12	m/58y	thigh/deep	10	R0	6, NER	
13	f/37y	shoulder/ sc, deep	15	NT	6, AWD	LN
14	m/82y	paravertebral/ cervical	NA	RX	NA	
15	f/46y	ankle	1	RM	220, AWD	4 rec, lung

Mos, months; Rec, recurrence; Met, metastases; Sc, subcutis; LN, lymph node; NER, no evidence of recurrence; AWD, alive with disease; DOD, death of disease; NA, not available; NT, no treatment; Perf, limb perfusion; Amp, amputation; R0, complete resection; RM, marginal resection; R1, resection with histological positive margins; R2, resection with macroscopically positive margins; RX, resection with unknown margins

Pathologic Findings

Grossly, the neoplasms were described as white or yellowish nodules, solid, gelatinous and also cystic in appearance. Hemorrhage and necrosis was seen in some of the lesions. The size range for MECs was 3.5 to 15 cm (mean, 6.7 cm) and for EMCs 1 to 15 cm (mean, 8.5 cm). In one EMC, the size was unknown.

Histologically, most cases demonstrated a (multi)-nodular configuration with expansive margins and an incomplete pseudo-capsule. Infiltrative margins were focally seen. MECs showed in most of the cases varying growth patterns such as trabecular, reticular, nested and solid. A pure trabecular pattern was seen in two cases. Six tumors were composed of different cell types, including spindle, epithelioid, plasmacytoid and/or clear cells. Round cell morphology associated with clear cells was observed in two cases and a pure epithelioid phenotype in two other cases (Figure 1). Osteoclast like giant cells were scattered in Cases 1 and 4. All cases exhibited moderate to severe nuclear atypia with vesicular or coarse chromatin (Figure 2). Prominent nucleoli were conspicuous in seven cases. Mitoses ranged from one to six per ten HPF. The matrix was (chondro)myxoid in 8 cases with pseudocystic changes in two cases. Five tumors showed stromal hyalinization. Areas of hemorrhage and tumor necrosis were observed in one and 2 cases respectively.

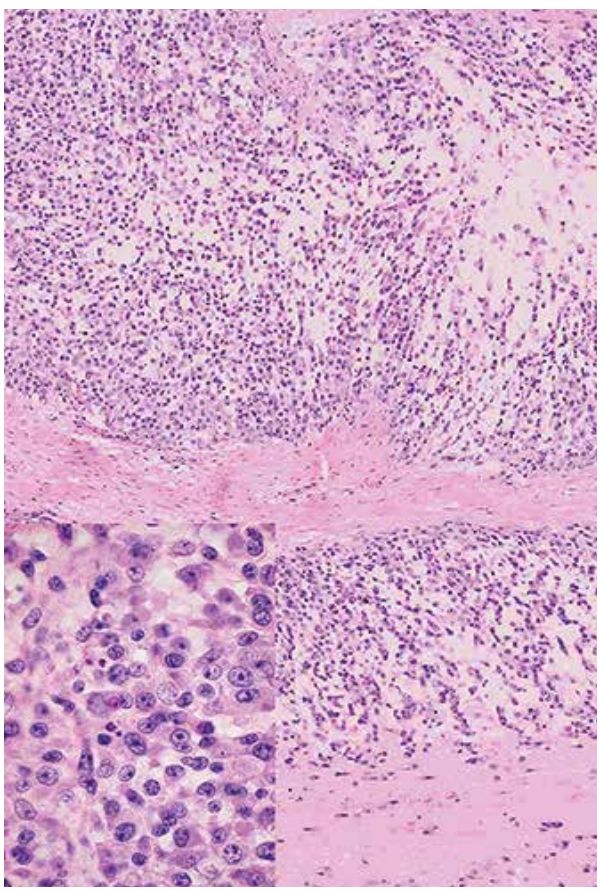

Figure 1 This case of MEC showed a reticular and cord like pattern of epithelioid cells set in a prominent myxoid matrix (Case 5)

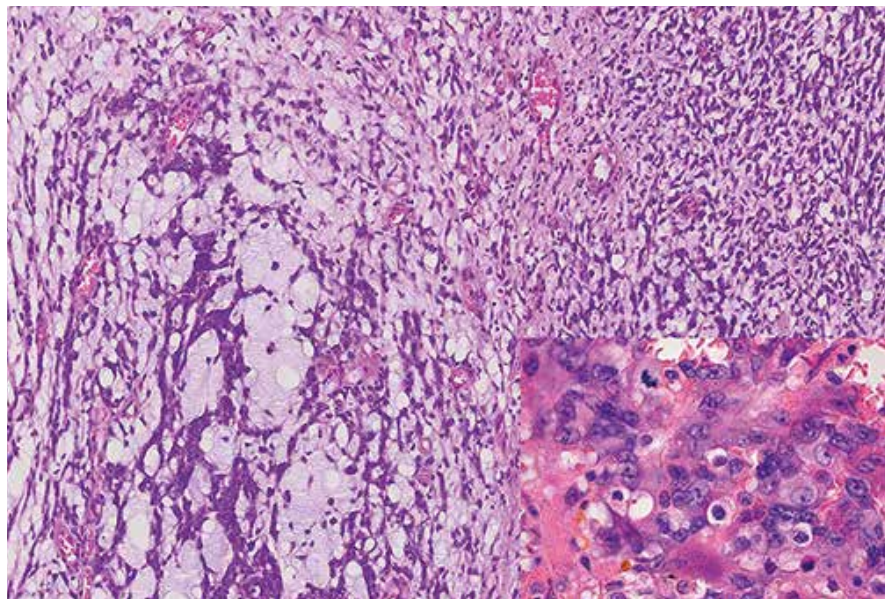

Figure 2 Note the nuclear pleomorphism, more often seen in MEC (Case 3)

All EMC cases had focally classical features with strands and cords of small, uniform round to spindle-shaped cells set in a prominent myxoid matrix. There were round to oval nuclei and limited deeply eosinophilic cytoplasm. The tumor nodules, separated by fibrous septa, showed often a peripheral cell condensation. In the cellular areas, sheets and nests of tumor cells were present. A trabecular and reticular-cystic pattern as well as loosely arranged cells occurred variably. The lesional cells were epithelioid and/or spindle-shaped and slightly pleomorphic (Figures 3 and 4). Case 13 showed increased pleomorphism, cytoplasmic vacuoles, multinucleated giant cells and prominent necrosis. In two cases, larger nuclei and prominent nucleoli were seen (cases 11, 12). Rhabdoid cytology was observed in Case 12. For all cases, the mitotic activity was very low ana did not exceed one mitosis/ten HPF. In the cellular areas, the myxoid matrix was scant or even absent.

Immunohistochemically, eight of ten (80%) myoepithelial carcinomas were, at least focally, positive for pan-keratin and four of seven (57%) for epithelial membrane antigen (EMA). The two cases negative for pankeratin expressed EMA. Two of eight cases showed nuclear immunoreactivity for p63 (25%). Alpha-smooth muscle actin (ASMA) was detected in six of eight (75%) and calponin in four of six cases (67%). A focal expression of S100 was seen in five of ten cases (50%). Glial fibrillary acidic protein (GFAP), in four cases performed, was negative in all four. In cEMCs, S100 and

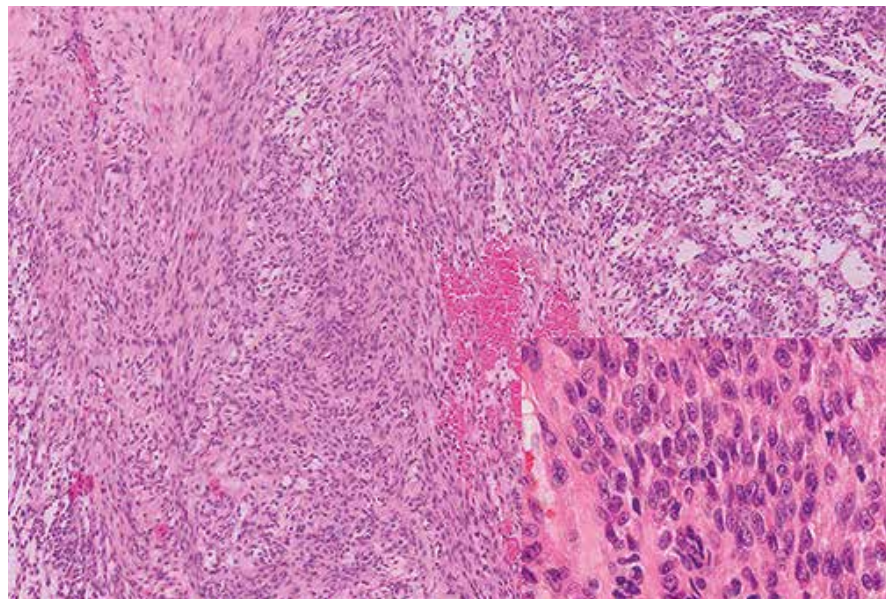

Figure 3 Monomorphic epithelioid and spindle-shaped cells in a cellular EMC (Case 11)

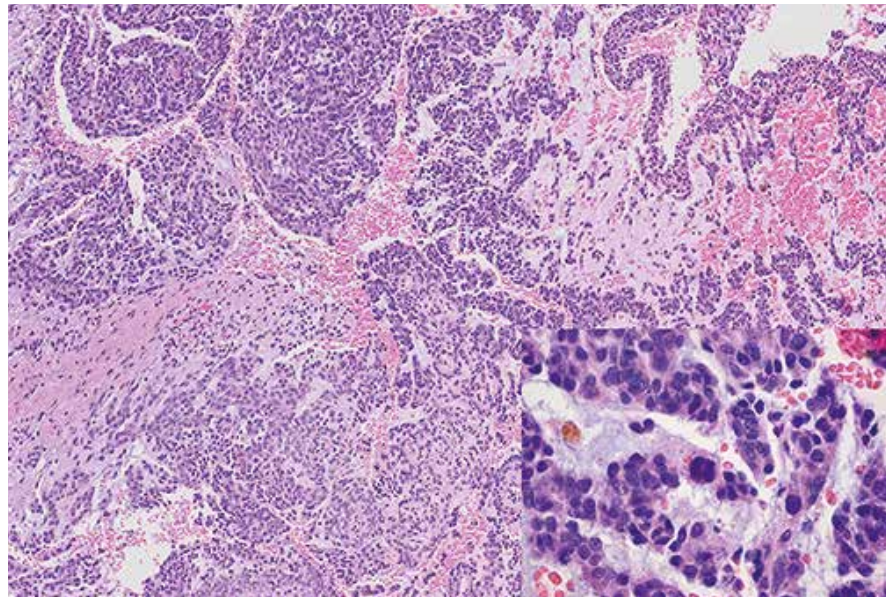

Figure 4 Solid and reticular arrangement of slightly polymorphic epitheloid cells in a cellular EMC

EMA were each expressed in two of five cases (40%). One of the mentioned S100-positive cases (Case 11) showed a broader pattern of marker expression with additional immunoreactivity for p63 (Figure 5), and focally for ASMA and GFAP. Pan-keratin was negative in all neoplasms (Table 3).

Table 3 Immunohistochemistry

Case	Pan-CK	EMA	p63	ASMA	S100	GFAP	Calponin
MEC							
1	-	+	-	f +	-	nd	+
2	+	nd	-	f +	f +	-	f +
3	f +	nd	-	f +	-	nd	f +
4	-	+	-	f +	-	-	nd
5	f +	-	nd	f+	-	nd	nd
6	+	nd	+	+	-	nd	nd
7	+	+	-	-	f +	-	-
8	+	-	nd	nd	+	-	+
9	+	-	-	-	f+	nd	-
10	+	+	+	nd	f+	nd	nd
	8/10	4/7	2/8	6/8	5/10	0/4	4/6
	80%	57%	25%	75%	50%	0%	67%
EMC							
11	-	-	+	f +	f +	f+	nd
12	-	-	-	-	f +	-	nd
13	-	f+	nd	-	-	-	nd
14	-	-	-	-	-	-	nd
15	-	f+	-	-	-	-	nd
	0/5	2/5	1/4	1/5	2/5	1/5	
	0%	40%	25%	20%	40%	20%	

f, focally
nd, not done

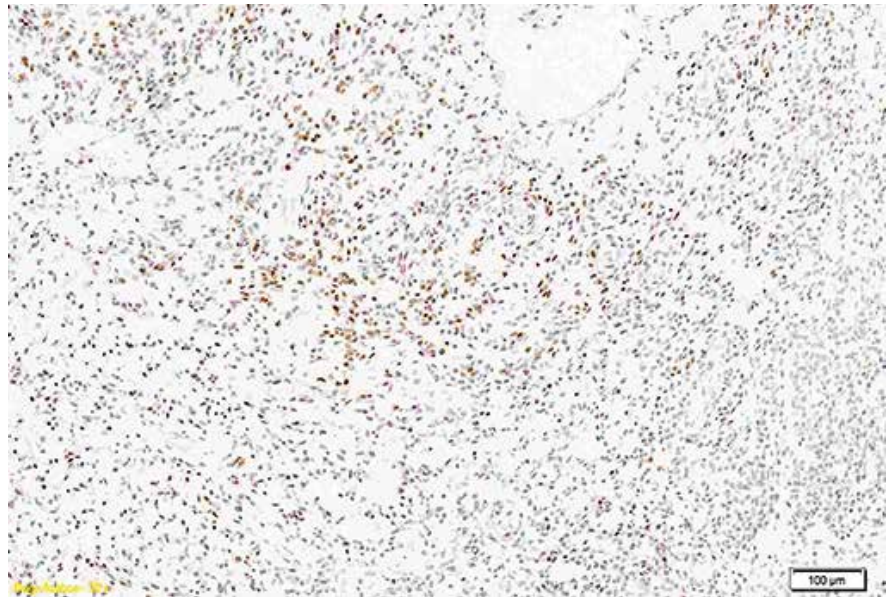

Figure 5 p63 was positive in one of the cellular EMC cases (Case 11)

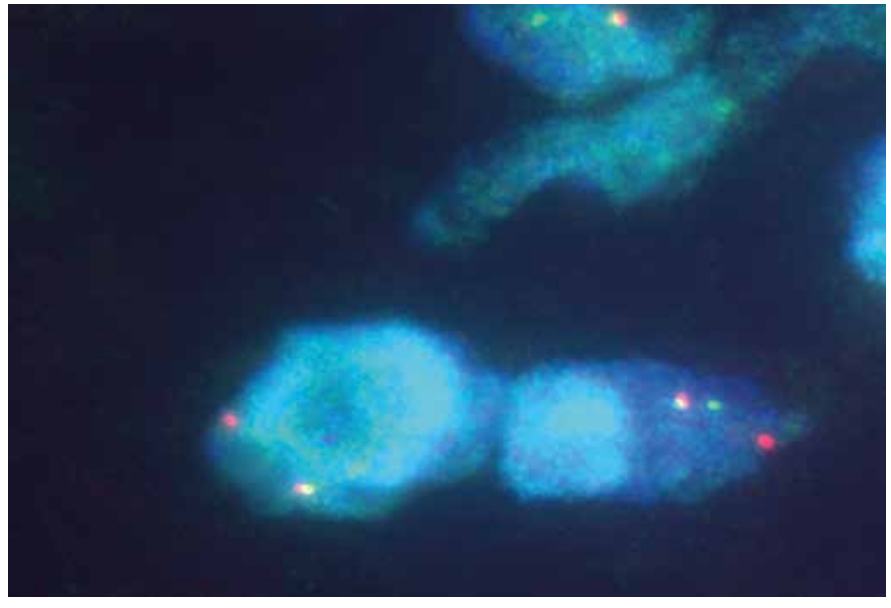

Figure 6 *NR4A3* rearrangement by FISH showing break-apart signal. This was observed in all EMC cases successfully tested

FISH and RT-PCR analysis

In three of nine (33%) MECs, a *EWSR1* rearrangement was observed by FISH. One case failed for analysis due to poor hybridisation. No tumor showed a *NR4A3* rearrangement. Aberrations of chromosome 9 were seen in 2 instances. One of them harbored a polysomy and one a heterozygous deletion. All EMCs successfully tested (four of five) exhibited a *NR4A3* rearrangement (Figure 6). One case failed for RT-PCR and *NR4A3*-FISH. Three cases showed a *EWSR1* rearrangement by FISH. In one of them a fusion of *TAF15-NR4A3* was additionally detected by RT-PCR. In Case 15, *NR4A3* was rearranged (by FISH) but a fusion with *EWSR1* or *TAF15* was not found (RT-PCR). *EWSR1-NR4A3* was evident in 1 neoplasm (Table 4).

Table 4 Molecular analyses (FISH*, RT-PCR)

Case	*EWSR1**	*NR4A3*/cep 9*	TAF15-NR4A3	EWSR1-NR4A3
MEC				
1	+	-		
2	+	heterozygote deletion chr. 9		
3	-	-		
4	-	-		
5	-	nd	-	-
6	+	-		
7	-	-		
8	-	polysomy chr. 9		
9	-	-		
10	x	x		
EMC				
11	+	nd	+	-
12	nd	nd	-	+
13	+	+	x	x
14	+	x	x	x
15	nd	+	-	-

+, rearrangement
-, no rearrangement
x, analysis failed
nd, not done

Discussion

The first myoepithelial tumor of the soft tissue was published by Stout and Gorman in 1959 in a series of cutaneous lesions.[30] In the largest series to date, moderate to severe atypia (vesicular or coarse chromatin, prominent, often large nucleoli, or nuclear pleomorphism) was determined as indicating malignancy.[2] The wide morphological and immunohistochemical diversity, presumably a result of the plasticity of myoepithelial cells, is the cause of the many differential diagnoses, which include most importantly the cellular variant of extraskeletal myxoid chondrosarcoma, furthermore are atypical/malignant ossifying fibromyxoid tumor, undifferentiated carcinoma, epithelioid MPNST, proximal type epithelioid sarcoma, and in cases with round cell morphology, Ewing sarcoma, myxoid/round cell liposarcoma and poorly differentiated synovial sarcoma are to be considered, at least in small samples.[2-4]

The occurence of myoepithelial tumors at different sites possibly reflects an aberrant gene expression pattern during oncogenesis rather than origin from a specific cell lineage.[3] This is supported by the evidence of *EWSR1* rearrangement in a subset of benign and malignant myoepithelial tumors of skin, soft tissue, bone and visceral locations (lung).[25,31] The hitherto identified fusion genes are *POU5F1*, *PBX1* and *ZNF444*.[23-25] Other more heterogenic genetic changes are also identified including recurrent aberrations of chromosome 9.[27,32-34] Recently, a *pleomorphic adenoma gene 1* (*PLAG1)* rearrangement was discovered in a subset of benign mixed tumors of skin and soft tissues (with well-formed ducts). It seems that this genetic abnormality excludes *EWSR1* rearrangement.[35] Whether malignant myoepithelial/mixed tumors possess similar features remains unresolved as yet.

When Enzinger designated extraskeletal myxoid chondrosarcoma as a distinct entity he already mentioned a resemblance with salivary gland type, mixed tumors of deep fascial region of thigh with reference to the report by Dutra in 1960.[5,36]

Classical cases of EMC have a typical histomorphology with uniform small round to spindle-shaped cells with deeply eosinophilic cytoplasm. The cells are arranged in a delicate network set in a copious myxoid stroma. Cellular variants, representing approximately one third of the cases, show greater morphologic heterogeneity, often resembling myoepithelial tumors.[2,4,5,8,10,11,25,33,37,38]

Both tumor types share features such as lobular or multinodular architecture, variable amounts of myxoid stroma and a reticular growth pattern. The stromal component in cellular areas can be poor or even absent. The cells variably epithelioid, round and spindle shaped. Rhabdoid cytology may also be present in both. Unlike myoepithelial carcinomas, moderate to severe nuclear atypia is much less common in cEMC and can therefore be a helpful discriminating sign. This was also a finding in our series. Ductular structures and metaplastic cartilage or bone, present in not more than 20% of soft tissue myoepithelial tumors, are not a feature of EMC.[2-8,10,38-41]

None of our MECs demonstrated one of these characteristics. Immunohistochemically, soft tissue myoepithelial tumors usually show expression of keratins (90-95%), EMA (60%), S100 (85%), GFAP (50%), calponin (90%), SMA (40%) and p63 (40%) in.[4,42] In contrast, EMCs are, often focally, positive for S100. GFAP, EMA, ASMA and keratins are expressed in the minority of cases, mostly with a focal staining pattern.[2,4,6,7,8,10,12,33,38-41] In our series,pan keratin was the most distinctive marker expressed in 80 % of MECs, but in none of the EMCs. Although our series is small, this result mirrors those by others.[8] p63, labeling myoepithelial cells at different sites, is at least focally positive in 40% of MECs and can be exceptionally positive in EMCs as we found in one of our cases.[42]

Loss of SMARCB1/INI1 is also an overlapping feature of the described entities and has been demonstrated in a subset of cases. Whereas underlying genetic alterations (homozygous deletion and frameshift mutation) have been detected in some EMCs, genetic aberrations have not been in myoepithelial carcinomas as yet.[3,39] Interestingly, the SMARCB1/INI1- negative EMC cases lack a typical major fusion gene transcript.[39] This raises the question whether these cases are more related to myoepithelial tumors.

EMCs are defined by specific reciprocal translocations, involving the pathognomonic *NR4A3*. The described fusion partners are, in decreasing frequency, *EWSR1, TAF15, TCF12* and *TGF*.[17-22]

By RT-PCR or FISH analysis, *NR4A3* rearrangement were found in all of our successfully tested EMCs. In one case, we detected besides a *TAF15-NR4A3* fusion an additional *EWSR1* rearrangement which is in line with another reported more complex rearranged case.[43] Furthermore, 33% of our myoepithelial carcinomas showed *EWSR1* rearrangement, consistent with the results by Antonescu et al.[25]

We did not find *NR4A3* rearrangement in any of the myoepithelial carcinomas, but as previously reported, changes of chromosome 9 as a recurrent aberration were seen in two of our cases.[27,34]

In summary, myoepithelial carcinoma of soft tissue and cellular extraskeletal myxoid chondrosaroma share clinical, morphological, immunohistochemical and genetic similarities. The pathognomic rearrangement of *NR4A3* and the general lack of keratin expression identify most cases of cEMC.

The seemingly poorer outcome especially in young patients with myoepithelial carcinomas and possible different treatment options make discrimination between these different entities necessary.

References

1. Kilpatrick SE, Hitchcock MG, Kraus MD, et al. Mixed tumors and myoepitheliomas of soft tissue: A clinicopathologic study of 19 cases with a unifying concept. Am J Surg Pathol 1997;21:13-22.
2. Hornick JL, Fletcher CDM. Myoepithelial tumors of soft tissue. A clinicopathologic and immunohistochemical study of 101 cases with evaluation of prognostic parameters. Am J Surg Pathol 2003;27:1183-1196.
3. Gleason BC, Fletcher CDM. Myoepithelial carcinoma of soft tissue in children: An aggressive neoplasm analysed in a series of 29 cases. Am J Surg Pathol 2007;31:1813-1824.
4. Gleason BC, Hornick JL. Myoepithelial tumours of skin and soft tissue: an update. Diagn Histopathol 2008;14:552-562.
5. Enzinger FM, Shiraki M. Extraskeletal myxoid chondrosarcoma. An analysis of 34 cases. Hum Pathol 1972;3:421-435.
6. Antonescu CR, Argani P, Erlandson RA, et al. Skeletal and extraskeletal myxoid chondrosarcoma: a comparative clinicopathologic, ultrastructural, and molecular study. Cancer 1998;83:1504-1521.
7. Lucas DR, Fletcher CDM, Adsay NV, Zalupski MM. High grade extraskeletal myxoid chondrosarcoma: a high-grade epithelioid malignancy. Histopathology 1999;35:201-208.
8. Meis-Kindblom JM, Bergh P, Gunterberg B, Kindblom LG. Extraskeletal myxoid chondrosarcoma: a reappraisal of its morphologic spectrum and prognostic factors based on 117 cases. Am J Surg Pathol 1999;23:636-650.
9. Oliveira AM, Sebo TJ, McGrory JE, et al. Extraskeletal myxoid chondrosarcoma: a clinicopathologic, immunohistocheicl, and ploidy analysis of 23 cases. Mod Pathol 2000;13:900-908.
10. Okamoto S, Hisaoka M, Ishida T, et al. Extraskeletal myxoid chondrosarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of 18 cases. Hum Pathol 2001;32:1116-1124.
11. Noguchi H, Mitsuhashi T, Seki K, et al. Fluorescence in situ hybridization analysis of extraskeletal myxoid chondrosarcomas using EWSR1 and NR4A3 probes. Hum Pathol 2010;41:336-342.
12. Hisaoka M, Hashimoto H. Extraskeletal myxoid chondrosarcoma: updated clinicopathological and molecular genetic characteristics. Pathol Int 2005;55:453-463.
13. Hachitanda Y, Tsuneyoshi M, Daimaru Y, et al. Extraskeletal myxoid chondrosarcoma in young children. Cancer 1988;61:2521-2526.
14. Saleh G, Evans HL, Ro JY, Ayala AG. Extraskeletal myxoid chondrosarcoma. A clinicopathologic study of ten patients with long-term follow-up. Cancer 1992;70:2827-2830.
15. Kawaguchi S, Wada T, Nagoya S, et al. Extraskeletal myxoid chondrosarcoma: a multi-institutional study of 42 cases in Japan Cancer 2003;97:1285-1292.
16. Drilon AD, Popat S, Bhuchar G, et al. Extraskeletal myxoid chondrosarcoma. A retrospective review from 2 referral centers emphasizing long-term outcomes with surgery and chemotherapy. Cancer 2008;113:3364-3371.
17. Sciot R, Dal Cin P, Fletcher C, et al. t(9;22) (q22-31;q11-12) is a consistent marker of extraskeletal myxoid chondrosarcoma: evaluation of three cases. Mod Pathol 1995;8:765-768.
18. Bjerkehagen B, Dietrich C, Reed W, et al. Extraskeletal myxoid chondrosarcoma: multimodal diagnosis and identification of a new cytogenetic subgroup characterized by t(9;17)(q22;q11). Virchows Arch 1999;435:524-530.
19. Sjögren H, Meis-Kindblom J, Kindblom LG, et al. Fusion of the *EWS*- related gene *TAF2N* to *TEC* in extraskeletal myxoid chondrosarcoma. Cancer Res 1999;59:5064-5067.
20. Sjögren H, Wedell B, Meis-Kindblom JM, et al. Fusion of the NH2-terminal domain of the basic helix-loop-helix protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9;15)(q22;q21). Cancer Res 2000;60:6832-6835.
21. Panagopoulos I, Mertens F, Isaksson M, et al. Molecular genetic characterization of the *EWS/CHN* and *RBP56/CHN* fusion genes in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer 2002;35:340-352.
22. Hisaoka M, Ishida T, Imamura T, Hashimoto H. TFG is a novel fusion partner of NOR1 in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer 2004;40:325-328.

23. Brandal P, Panagopoulos I, Bjerkehagen B, et al. Detection of a t(1;22)(q23;q12) translocation leading to an EWSR1-PBX1 fusion gene in a myoepithelioma. Genes Chromosomes Cancer 2008;47:558-564.
24. Brandal P, Panagopoulos I, Bjerkehagen B, Heim S. t(19;22)(q13;12) Translocation leading to the novel fusion gene EWSR1-ZNF444 in soft tissue myoepithelial carcinoma. Genes Chromosomes Cancer 2009;48:1051-1056.
25. Antonescu CR, Zhang L, Chang NE, et al. *EWSR1-POU5F1* fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone and visceral lesions showing common involvement of EWSR1 gene rearrangement. Genes Chromosomes Cancer 2010;49:1114-1124.
26. Fletcher CDM, Unni K, Mertens F (eds) World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft tissue and Bone. IARC press, Lyon 2002; pp 198-199, pp 213-215.
27. van den Berg E, Zorgdrager H, Hoekstra HJ, Suurmeijer AJH. Cytogenetics of a soft tissue malignant myoepithelioma. Cancer Genet Cytogenet 2004;151:87-89.
28. Bell E, Van der Biezen JJ, Werker PM. Parachordoma: a very rare tumor of the hand. J Hand Surg Eur Vol 2009;34:814-816.
29. Hantschke M, Mentzel T, Rütten A, et al. Cutaneous clear cell sarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of 12 cases emphasizing its distinction from dermal melanoma. Am J Surg Pathol 2010;34:216-220.
30. Stout AP, Gorman JG. Mixed tumors of the skin of the salivary gland type. Cancer 1959;12:537-543.
31. Flucke U, Palmedo G, Blankenhorn N, et al. *EWSR1* gene rearrangement occurs in a subset of cutaneous myoepithelial tumors: a study of 18 cases. Mod Pathol 2011;11:1444-1450.
32. Pauwels P, Dal Cin P, Roumen R, et al. Intramuscular mixed tumour with clonal chromosomal changes. Virchows Arch 1999;434:167-171.
33. Folpe AL, Agoff SN, Willis J, Weiss SW. Parachordoma is immunohistochemically and cytogenetically distinct from axial chordoma and extraskeletal myxoid chondrosarcoma. Am J Surg Pathol 1999;23:1059-1067.
34. Hallor KH, Teixeira MR, Fletcher CDM, et al. Heterogeneous genetic profiles in soft tissue myoepitheliomas. Mod Pathol 2008;21:1311-1319.
35. Bahrami A, Dalton JD, Krane JF, Fletcher CDM. A subset of cutaneous and soft tissue mixed tumors are genetically linked to their salivary gland counterpart. Genes Chromosomes Cancer 2011;doi: 10.1002/gcc.20938
36. Dutra FR. Mixed tumor, salivary gland type, of deep fascial region of thigh. Arch Path 1960;70:562-564.
37. Fletcher CDM. Soft tissue tumors. In: Fletcher CDM (ed). Diagnostic histopathology of tumors. 3rd edn. Churchill Livingstone Elsevier, Philadelphia, 2007;pp 1573-1574.
38. Dei Tos AP, Wadden C, Fletcher CDM. Extraskeletal myxoid chondrosarcoma: an immunohistochemical reappraisal of 39 cases. Appl Immunohistochem 1997;5:73-77.
39. Kohashi K, Oda Y, Yamamoto H, et al. SMARCB1/INI1 protein expression in round cell soft tissue sarcomas associated with chromosomal translocation involving EWS: a special reference to SMARCB1/INI1 negative variant extraskeletal myxoid chondrosarcoma. Am J Surg Pathol 2008;32:1168-1174.
40. Oshiro Y, Shiratsuchi H, Tamiya S, et al. Extraskeletal myxoid chondrosarcoma with rhabdoid features, with special reference to its aggressive behavior. Int J Surg Pathol 2000;8:145-152.
41. Abramovici LC, Steiner GC, Bonar F. Myxoid chondrosarcoma of soft tissue and bone: a retrospective study of 11 cases. Hum Pathol 1995;26:1215-1220.
42. Jo VY, Fletcher CDM. P63 immunohistochemical staining is limited in soft tissue tumors. Am J Clin Pathol 2011;236:762-766.
43. Turc-Carel C, Dal Cin P, Rao U, et al. Recurrent breakpoints at 9q31 and 22q12.2 in extraskeletal myxoid chondrsarcoma. Cancer Genet Cytogenet 1988;30:145-150.

Chapter 5

Myxoid epithelioid sarcoma: a diagnostic challenge. A report on six cases

Histopathology 2010 Nov;57(5):753-9.

Abstract

Epithelioid sarcoma (ES) is a distinct sarcoma-type with a specific morphology and immunophenotype. Whereas focal myxoid change does occur, to our knowledge only two cases of ES with diffuse myxoid stroma have been reported previously. To characterize more clearly the myxoid variant of ES, we describe six additional cases and discuss the differential diagnoses.

Cases were retrieved from the authors' files and studied histologically, immunohistochemically and by molecular methods. The age of the patients, four females and two males, ranged from 16 to 74 years (median: 33 years). The neoplasms arose in an extremity (two cases), the abdominal wall, groin, perineum and shoulder (one case each). Histologically, four cases were of the conventional type, and two were of the proximal type and the immunophenotype was typical for ES. The tumor stroma, however, revealed prominent myxoid changes, ranging from 50 to 90% (median: 75%). Only one of the proximal type ES showed a *SMARCB1* mutation, whereas the other tumors showed no mutation.

The myxoid variant of ES represents a diagnostic challenge and may be confused with other myxoid benign and malignant neoplasms. The main differential diagnosis is myoepithelioma of the skin and soft tissue.

Introduction

In the first description of 62 cases of epithelioid sarcoma (ES) in 1970 by Enzinger, extensive extracellular mucinous material was present in five of 15 cases, that had been stained for mucopolysaccharides.[1] Nevertheless, myxoid stromal change in ES was described as a rare occurrence in subsequent studies.[2-4]

In contrast to ES, benign or malignant myoepithelial neoplasms of skin and soft tissues often show, at least focally, prominent myxoid stroma.[5-9] Like ES, cases of myoepithelioma tend to occur on the extremities of adolescents and adults and may show overlapping morphological features with ES such as a nodular proliferation of spindled and epithelioid tumor cells. More rarely, myoepithelioma and ES may occur in children.[1,7-11] The immunophenotype of myoepitheliomas and ES shares similarities with simultaneous expression of epithelial markers and vimentin, although actins are expressed more rarely in ES. Discriminating markers for myoepitheliomas are S-100, GFAP and calponin, being positive in the majority of cases.[6-9] Approximately 50-67% of ES are positive for CD34 which is not seen in myoepitheliomas.[3,5-9,12-16] Most cases of ES (> 80%) show loss of SMARCB1 protein expression, and this phenomenon is also described in a subset of myoepithelial carcinomas.[9,16-18]

In this study we describe six cases, the largest series so far, of the myxoid variant of the distal and proximal type ES, and discuss the differential diagnosis, with special emphasis on the distinction from benign and malignant myoepithelioma of skin and soft tissue.

Material and methods

The cases were retrieved from the files of the Department of Pathology, Radboud University Medical Centre Nijmegen (Nijmegen, The Netherlands), of the Dermatopathologie Bodensee (Friedrichshafen, Germany) and from the consultation files of one of the authors (TM). One case was selected from PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands. Slides were reviewed and the diagnosis of ES was based on histological and immunohistochemical criteria according to WHO classification.[22] Cases in which 50% or more of the tumor area showed myxoid change were included in the study. The specimens had been obtained by surgical excision, fixed in 10% buffered formalin, and processed for paraffin embedding. Four-µm-thick sections were stained with haematoxylin and eosin, and immunohistochemically by the labeled streptavidin-biotin technique using commercially available antibodies; appropriate positive and negative controls were used in each case. Antibodies, their sources and dilutions are summarized in Table 1.

Table 1 Panel of antibodies used in this study

Antigen	Clone	Dilution	Source
Vimentin	V9	1:150	Dako
Pan-cytokeratin	MNF116	1:100	Dako
Pan-cytokeratin	AE1/3	1:50	Dako
CD34	HPCA-1	1:100	Becton & Dickinson
SMA	1A4	1:300	Dako
BAF 47	25	1:250	BD Transduction Laboratories
S-100	Polyclonal	1:2000	Dako
Calponin	CALP1	1:400	Dako
EMA	E29	1:3000	Dako
P63	4A4	1:400	Dako
GFAP	GA-5	1:200	Biogenix

Genomic DNA was extracted from the paraffin embedded specimens of all six cases by use of commercially available kits (Qiagen, Venlo, The Netherlands). *SMARCB1* mutation analysis was performed as described previously.[23] The clinical information were obtained from the referring clinicians and pathologists.

Results

Clinical findings

Details are summarized in Table 2. The neoplasms arose in four females and two males. The age of the patients ranged from 16 to 74 years (median: 33 years). Anatomic sites included the extremities (two cases), the abdominal wall, perineum, groin, shoulder (one case each). The patients were treated by wide excision or reexcision with tumor free margins in all cases. Local recurrences were seen in three cases, two of the distal and one of the proximal type. The two recurring distal type neoplasms showed two and four recurrences respectively, and subsequently developed lymph node metastases.

Pathological features

The size of the neoplasms ranged from 1.0 to 4.0 cm (median: 1.8 cm). Sites of involvement were the dermis and subcutis of the abdominal wall, schoulder and hand and soft tissue of the lower leg (distal type), and subcutis of the groin, and vulva (proximal type).

Table 2 Clinical and morphological details of six cases of myxoid ES

Case	Sex	Age (years)	Location	Type	Size (cm)	Amount of myxoid changes (%)	Recurrences (n)	Metastases
1	M	48	abdominal wall (dermis, subcutis)	distal	1.6	80	2	lymph node groin
2	F	22	vulva (subcutis)	proximal	1.5	70	NA	NA
3	F	16	lower leg (soft tissue)	distal	2.0	90	NA	NA
4	M	74	shoulder, (dermis, subcutis)	distal	4.0	50	no	No
5	F	41	hand (subcutis)	distal	1.0	50	4	lymph nodes axilla
6	F	25	groin (subcutis)	proximal	4.0	90	1	NA

Microscopically, in all tumors a (multi)nodular growth with infiltrative margins were seen (Figure 1). The tumor cells were arranged in nests and cords, with the myxoid stromal background. A reticular growth pattern was observed in three cases (two of the distal type and one of the proximal type) (Figures 2 and 3). Classical solid areas were seen in all cases (Figures 1 and 4). The epithelioid tumor cells contained abundant eosinophilic cytoplasm and round, vesicular nuclei. Prominent nucleoli were seen variably. In all four cases of the distal type, scattered spindle-shaped tumor cells were noted. In two cases plasmocytoid cells were also recognized (Figure 5). Centrally the tumors showed hyalinization (4 cases) and/or necrosis (3 cases). The two cases of proximal type were composed of larger epithelioid and rhabdoid tumor cells (Figures 3 and 4).

Necrosis was seen in one of the proximal type, and in all but one of the distal ES. An inflammatory reaction was more prominent in the cases of the distal type. The mitotic rate ranged from 12 to 24 mitoses per 2mm² in the cases of the proximal type and from 3 to 11 mitoses per 2mm² in the cases of the distal type.

Two cases were 90% myxoid, and one case each showed 80% and 70% myxoid stromal change, whereas in two cases 50% myxoid areas were observed (Table 2).

Immunohistochemically, tumor cells in all cases stained strongly positive for vimentin, pan-cytokeratin (Figure 6) and EMA. In addition, CD34 was positive in two cases (Figure 7), and expression of ASMA was present focally in two cases. Weak and

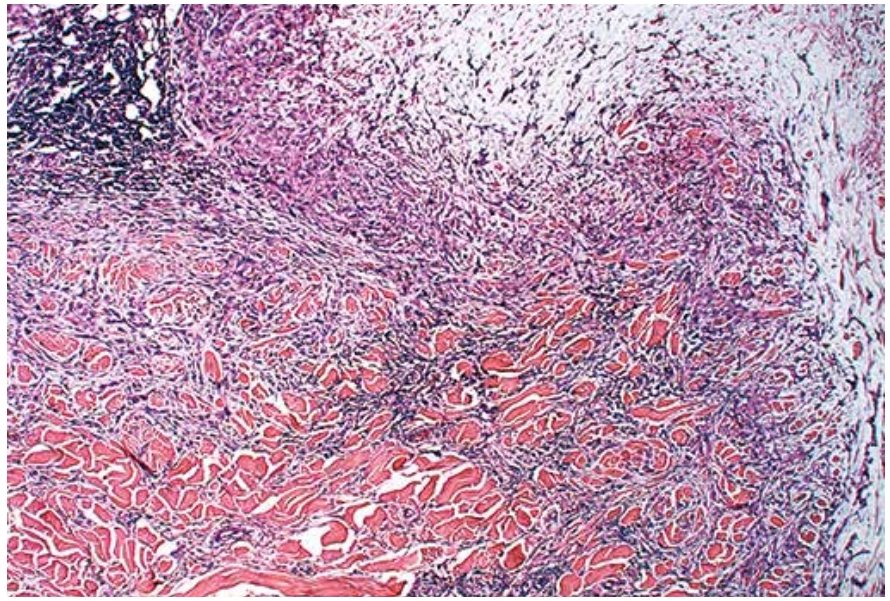

Figure 1 Classical features in one of the distal type ES (case 1) with nodular architecture and inflammatory reaction; myxoid background is also seen

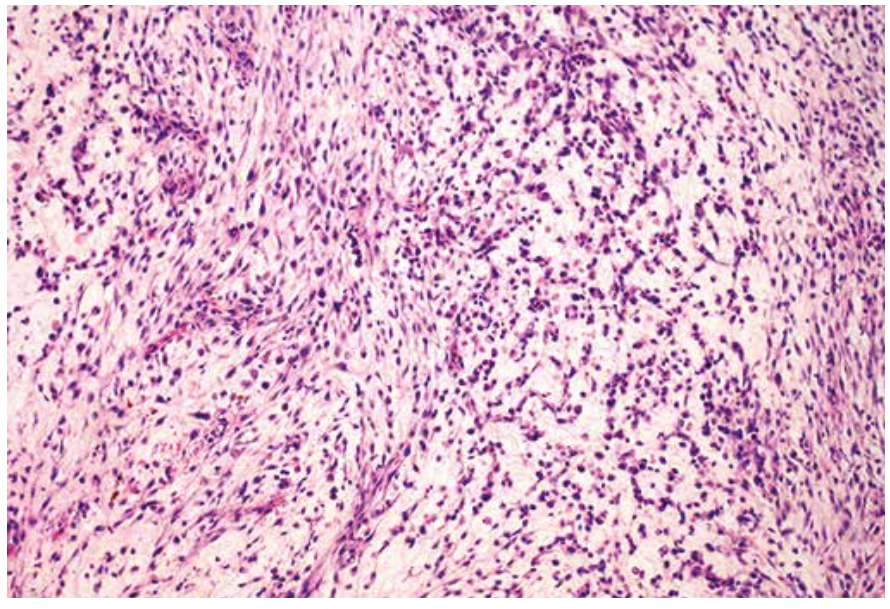

Figure 2 Epithelioid and spindle-shaped tumour cells in case 3 are arranged in cords given a reticular growth pattern. Note the prominent myxoid stroma

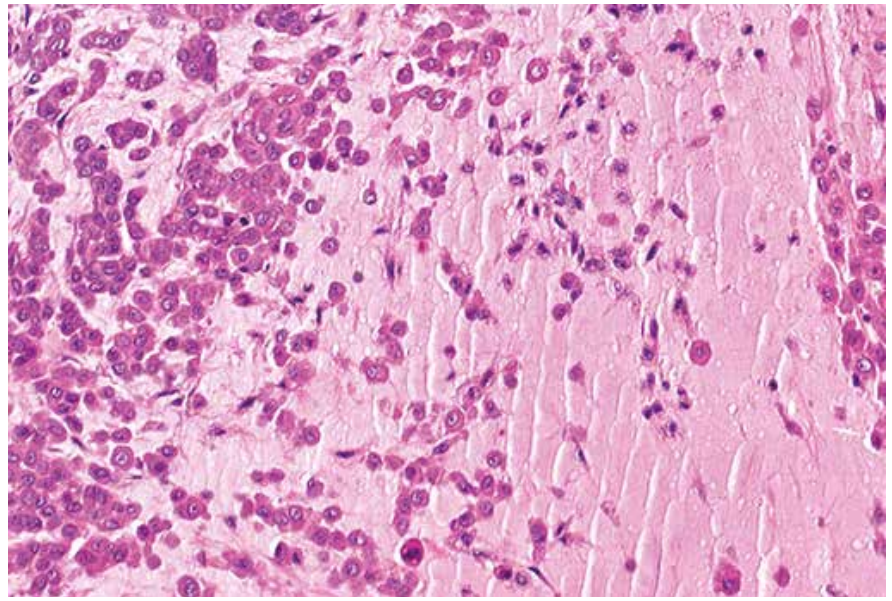

Figure 3 This neoplasm of the proximal type is composed of nests and cords of enlarged tumour cells with rhabdoid features (case 2)

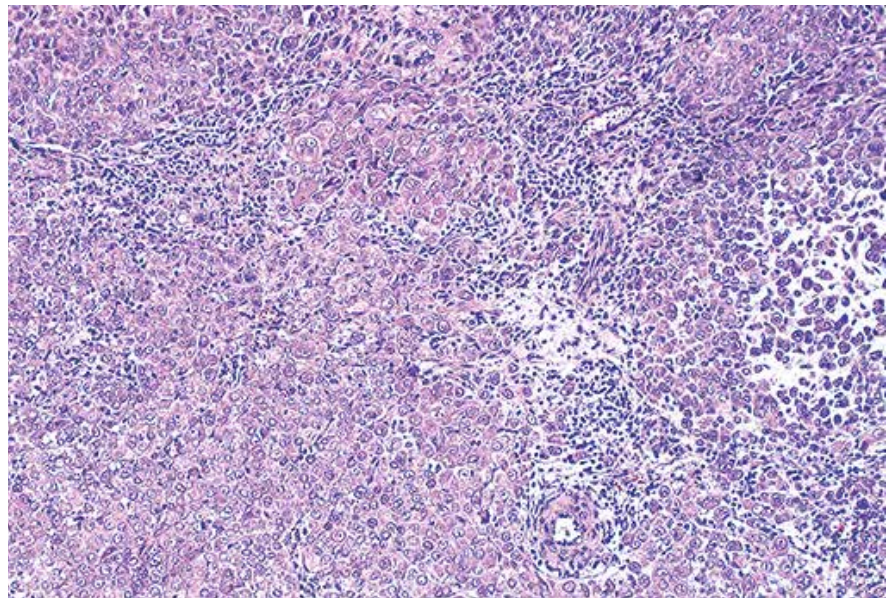

Figure 4 Predominantly solid areas in a proximal type ES (case 6)

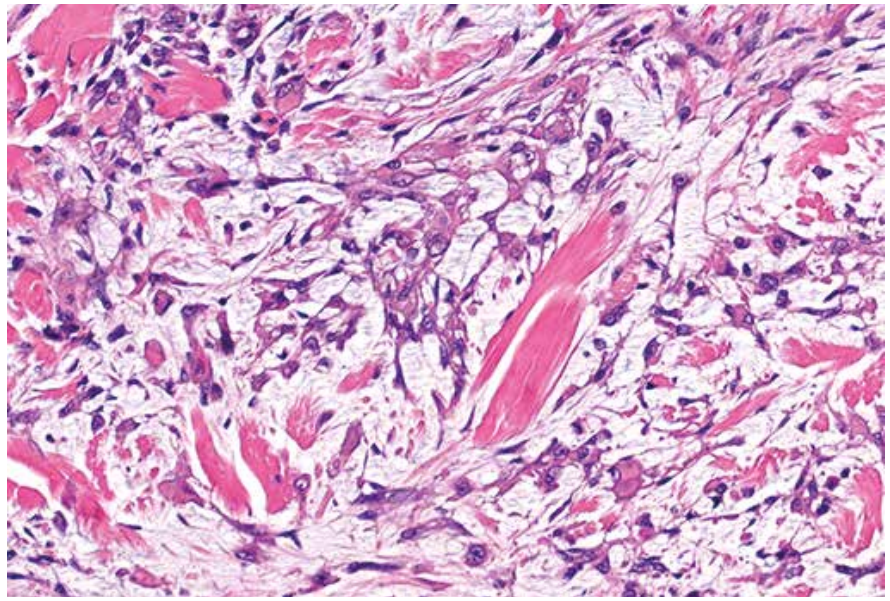

Figure 5 Epithelioid and spindle tumour cells set in a prominent myxoid stroma; scattered plasmocytoid cells are also present (case 4)

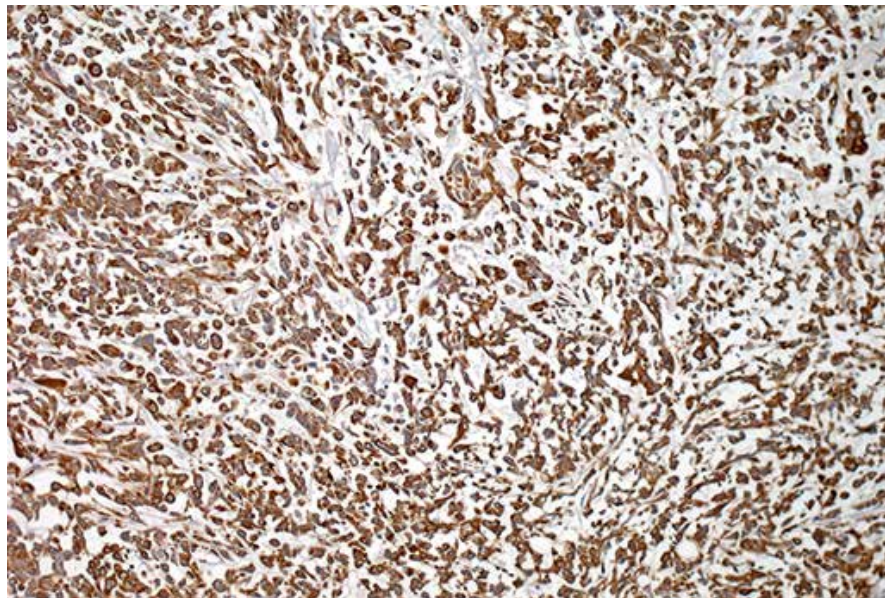

Figure 6 Pan-cytokeratin expression was a characteristic feature of all cases (case 6)

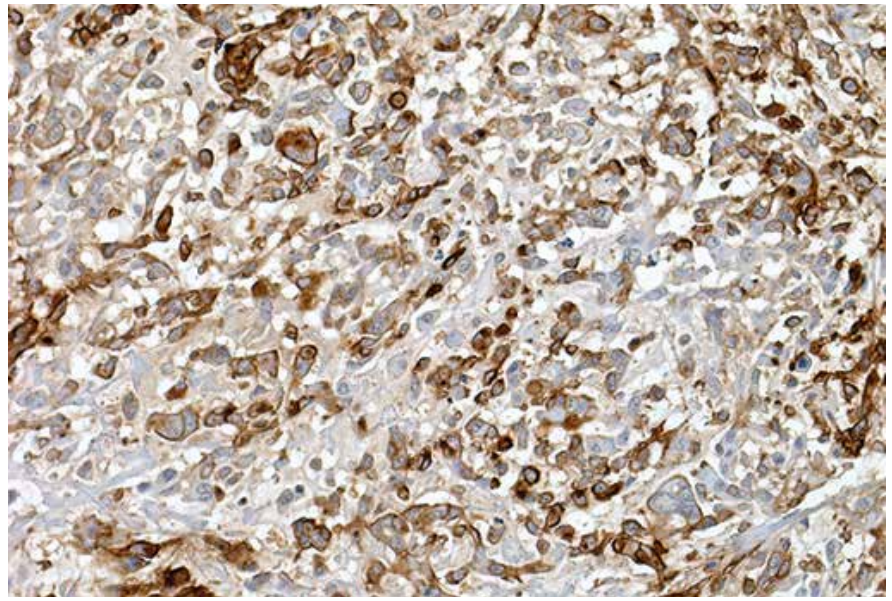

Figure 7 CD34 positivity was observed in two cases (case 6)

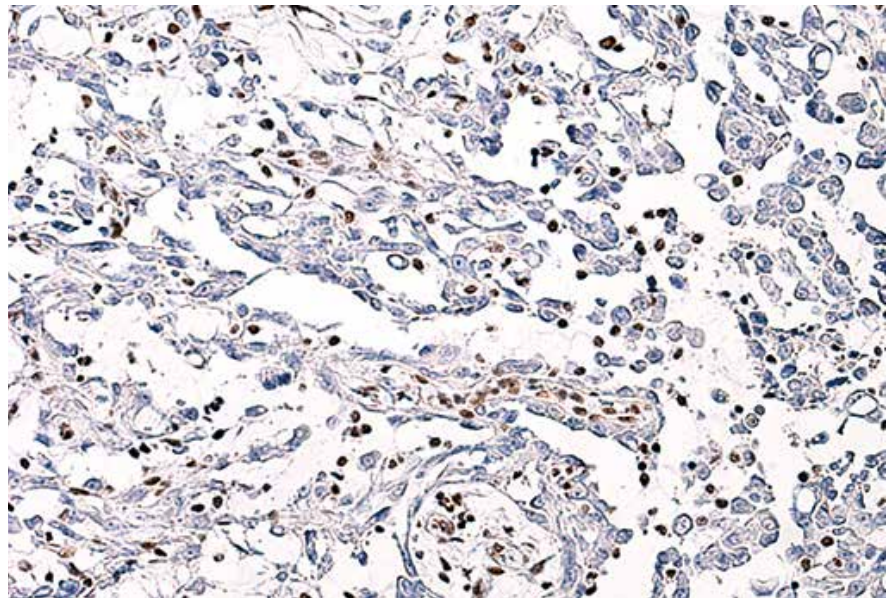

Figure 8 All cases showed loss of SMARCB1 protein expression (case 6)

focal positivity of calponin was seen in three cases. SMARCB1 (BAF47) was absent in all six cases (Figure 8). None of the neoplasms expressed S-100 protein, GFAP, or p63 (Table 3).

SMARCB1 gene status

All tumors were investigated for the presence of mutations of the *SMARCB1* gene. In one tumor a mutation in exon 6 was recognized, which we have reported previously.[20] In this case of proximal type, localized in the vulva, we identified a c.769C>T transition resulting in the generation of an in-frame stopcodon (CAG->TAG, p.Q257X) in the transcript and premature termination of SMARCB1INI1 protein synthesis. In accordance with the two-hit model for tumor genesis, we noted considerable loss of the wild-type copy of this gene in the tumor. None of the other cases had mutation of *SMARCB1*.

Table 3 Immunohistochemical results of six cases of myxoid ES

Case	Vimentin	CK	CD34	EMA	SMA	BAF47	S100	GFAP	P63	Calponin
1	+	+	+	+	-	-	-	-	-	-
2	+	+	-	+	-	-	-	-	-	-
3	+	+	-	+	+ focal	-	-	-	-	+ focal,weak
4	+	+	-	+	-	-	-	-	-	+ focal,weak
5	+	+	-	+	+ focal	-	-	-	-	+ focal,weak
6	+	+	+	+	-	-	-	-	-	-

Discussion

Myxoid tumors of soft tissue encompass a heterogenous group of lesions characterized by a marked abundance of extracellular mucoid (myxoid) matrix. Thus, they demonstrate significant variability in their biological behavior thus including indolent tumors, tumors with a tendency to recur locally but not metastasize, and malignant tumors.[24] Although in classical ES myxoid stromal changes are well known, only two cases of ES with diffuse myxoid background have been described so far.[1,4,14,25]

We report an additional series of six cases of myxoid ES and discuss their morphological, immunohistochemical and molecular features, and the differential diagnosis to other benign and malignant neoplasms of skin and soft tissues.

The reported neoplasms, two of the proximal and four of the distal type, were localized in the dermis, subcutis and deep soft tissues, and showed classical morphological and immunohistochemical features of ES. In addition, prominent myxoid stromal changes were seen in all cases and tumor cells were often arranged in cords and nests; a somewhat reticular architecture was seen in three cases. Our findings are comparable to those described earlier for two distal type myxoid ES.[25]

All cases were negative for SMARCB1 protein expression. This confirms recently published studies, showing loss of SMARCB1 protein expression in more than 80% of cases of ES.[16-18,20] Only one of our proximal-type ES showed a *SMARCB1* mutation, whereas the other tumours had no mutation. The low frequency of mutations affecting the *SMARCB1* gene in our series of ES is comparable to others and suggests that the expression of SMARCB1 in this tumor type is down-regulated by alternative mechanisms, such as epigenetic modification.[18,20]

The main differential diagnosis of myxoid ES is myoepithelioma of skin and soft tissues. These neoplasms also arise predominantly in the extremities of (young) adults and children. Because of the plasticity of myoepithelial cells, they show heterogeneous morphological features, consisting of spindled, epithelioid, plasmocytoid and clear cells often set in a (chondro)myxoid or collagenous/hyalinized stroma.[5-8] Hence, there is morphological overlap with myxoid ES, showing predominantly epithelioid and spindle shaped cells. Ductal structures in myoepitheliomas are of help in the distinction of both entities. This feature was not observed in our cases. Futhermore, all of our cases showed also classical morphology of ES in at least 10% of tumor. The distal type cases in our series showed a granulomatous appearance and necrosis. Moreover, none of the tumors of our series expressed S-100 protein, which was seen in 87% to 93% of myoepithelial neoplasms.[7,8] The presence of pankeratin and EMA was 100% in our cases as expected in ES, while AE1/AE3 is positive in only 77% and EMA in only 63% of myoepithelial neoplasms.[7] Smaller series showed similar results.[8,9] The focal staining for ASMA in two and calponin among three of our cases was very weak and not comparable to that is seen in myoepithelial neoplasms.[5-9] Two of our cases showed discriminating positivity for CD34, which concurs with its reported positivity in approximately 50 % of ES.[3,13, 14,16] We observed negative staining results for GFAP and p63, analogous to the results of others, whereas positivity has been described in approximately 46 - 100% and 7 - 27% of myoepithelial neoplasms, respectively.[6-9,15]

As in ES, SMARCB1 protein expression in myoepithelial carcinomas may be absent, as has been shown recently,[9,17] the genetic background of which is still unknown.

Epithelioid haemangioendothelioma (EHE) of skin and soft tissues may also enter the differential diagnosis of myxoid ES. This distinct malignant vascular tumor, occurring most commonly in middle-aged patients, has a wide range of localization. Tumors of the skin are very rare and both single and multiple lesions have been

described; the last is seen typically in progressive ES. Histologically, EHE shows a (multi) nodular architecture and is composed of short strands, cords or small clusters of epithelioid to slightly spindled cells with an eosinophilic cytoplasm and vesicular nuclei. In some cases, intracytoplasmatic vacuoles, usually a prominent feature in EHE, could be in some cases very subtle and are recognized occasionally in ES due to lipid droplets. The typically myxohyaline stroma shows, variably, haemorrhage and/or haemosiderin deposits. Discriminating immunohistochemical features are positivity for specific endothelial markers, as CD31 (with a strong membranous reaction) and Fli-1 (with nuclear staining) in EHE, whereas EMA, usually positive in ES, is rarely detected in EHE. Cytokeratin and CD34, variably positive in both tumors, are not useful in the differential diagnosis.[26-29]

Another differential diagnosis of ES represents extraskeletal myxoid chondrosarcoma (EMC). EMC typically shows a multinodular growth pattern with interlacing cords of round or plump-spindled tumor cells set in a chondomyxoid background. In contrast to ES, the cells of EMC are less epithelioid and the architecture is more homogeneous. A cellular high grade variant and rhabdoid features is also described, showing a greater degree of morphological overlap especially with proximal type ES.[30,31] The rhabdoid features in EMC were associated with loss of SMARCB1/INI1 expression and partly with genetic alteration in *SMARCB1/INI*.[31] If immunohistochemistry is required for a definitive diagnosis, S-100 is expressed in a minority of cases, and epithelial and myogenic markers are usually absent.[7,32] Typical translocations, t(9;22) (q22;q12), t(9;17) (q22;q11), and t(9;15) (q22;q21), provide proof proof of this sarcoma.[33,34]

Recently, an epithelioid variant of myxofibrosarcoma has been described composed mainly of epithelioid fibroblastic cells set in a myxoid stroma. In addition pseudolipoblasts and numerous elongated, curvilinear blood vessels are present, mostly seen in the hypocellular myxoid areas.[35] This tumor usually arises in elderly patients, lacks the expression of epithelial markers and does not show loss of SMARCB1 protein expression.

In summary myxoid ES represents a rare variant of ES and prominent myxoid stromal changes are seen in both the distal and proximal types. Correct diagnosis can be problematic, particularly in small biopsy specimens, and cases of benign and malignant myoepithelioma, extraskeletal myxoid chondrosarcoma and epithelioid myxofibrosarcoma have to be distinguished from myxoid ES.

References

1. Enzinger FM. Epithelioid sarcoma: a sarcoma simulating a granuloma or a carcinoma. Cancer 1970;26:1029-1041.
2. Evans HL, Baer SC. Epithelioid sarcoma: a clinicopathologic and prognostic study of 26 cases. Semin Diagn Pathol 1993;10:286-291.
3. Hasegawa T, Matsuno Y, Shimoda T, Umeda T, Yokoyama R, Hirohashi S. Proximal-type epithelioid sarcoma: a clinicopathologic study of 20 cases. Mod Pathol 2001;14:655-663.
4. Fisher C. Epithelioid sarcoma of Enzinger. Adv Anat Pathol 2006;13:114-121.
5. Kilpatrick SE, Hitchcock MG, Kraus MD, Calonje E, Fletcher CDM. Mixed tumors and myoepitheliomas of soft tissue: A clinicopathologic study of 19 cases with a unifying concept. Am J Surg Pathol 1997;21:13-22.
6. Kutzner H, Mentzel T, Kaddu S, Soares LM, Sangueza OP, Requena L. Cutaneous myoepithelioma: an underecognized cutaneous neoplasm composed of myoepithelial cells. Am J Surg Pathol. 2001;25:348-355.
7. Hornick JL, Fletcher CDM. Myoepithelial tumors of soft tissue. A clinicopathologic and immunohistochemical study of 101 cases with evaluation of prognostic parameters. Am J Surg Pathol 2003;27:1183-1196.
8. Hornick JL, Fletcher CDM. Cutaneous myoepithelioma: A clinicopathologic and immunohistochemical study of 14 cases. Hum Pathol 2004;35:14-24.
9. Gleason BC, Fletcher CDM. Myoepithelial carcinoma of soft tissue in children: An aggressive neoplasm analysed in a series of 29 cases. Am J Surg Pathol 2007; 31:1813-1824.
10. Chase DR, Enzinger FM. Epithelioid sarcoma: Diagnosis, prognostic indicators and treatment. Am J Surg Pathol 1985;9:241-263.
11. Casanova M, Ferrari A, Collini P et al. Epithelioid sarcoma in children and adolescents: A report from the Italian Soft Tissue Sarcoma Committee. Cancer 2006;106:708-717.
12. Mirra JM, Kessler S, Butha S, Eckhardt J. The fibroma-like variant of epithelioid sarcoma. Cancer 1992; 69:1382-1395.
13. Guillou L, Wadden C, Coindre JM, Krausz T, Fletcher CDM. Proximal-type epithelioid sarcoma, a distinctive aggressive neoplasm showing rhabdoid features: clinicopathologic, immunohistochemical, and ultrastructural study of a series. Am J Surg Pathol 1997;21:130-146.
14. Miettinen M, Fanburg-Smith JC, Virolainen M, Shmookler BM, Fetch JF. Epithelioid sarcoma: an immunohistochemical analysis of 112 classical and variant cases and a discussion of the differential diagnosis. Hum Pathol 1999;30:934-942.
15. Laskin WB, Miettinen M. Epithelioid sarcoma. New insights based on an extended immunohistochemical analysis. Arch Pathol Lab Med 2003;127:1161-1168.
16. Chbani L, Guillou L, Terrier P et al. Epithelioid sarcoma. A clinicopathologic and immunohistochemical analysys of 106 cases from the French Sarcoma Group. Am J Clin Pathol 2009;13:222-227.
17. Hornick JL, Dal Cin P, Fletcher CDM. Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. Am J Surg Pathol 2009; 33:542-550.
18. Kohashi K, Izumi T, Oda Y, et al. Infrequent SMARCB1/INI1 gene alteration in epithelioid sarcoma: a useful tool in distinguishing epithelioid sarcoma from malignant rhabdoid tumor. Hum Pathol 2009;40:349-355.
19. Modena P, Lualdi E, Facchinetti F, et al. SMARB1/INI1 tumor suppressor gene is frequently inactivated in epithelioid sarcomas. Cancer Res 2005;65:4012-4019.
20. Flucke U, Mentzel T, Slootweg PJ, Pauwels P, Hulsebos TJM. Direct evidence of mutational inactivation of *SMARCB1/INI1* in epithelioid sarcoma. Hum. Pathol. 2009;40:1361-1362.
21. von Hochstetter AR, Meyer VE, Grant JW, Honegger HP, Schreiber A. Epithelioid sarcoma mimicking angiosarcoma: the value of immunohistochemistry in the differential diagnosis. Virchows Arch A Pathol Anat Histopathol 1991;418:271-278.
22. Guillou L, Kaneko Y. Epithelioid sarcoma. In Fletcher CDM, Unni KK and Mertens F eds. Pathology and Genetics of Tumours of Soft Tissue and Bone. World Health Organisation Classification of Tumours. Lyon, France: IARC Press; 2002; 205-207.

23. Hulsebos TJM, Plomp AS, Wolterman RA, Robanus-Maandag EC, Baas F, Wesseling P. Germline mutation of *INI1/SMARCB1* in familial schwannomatosis. Am J Hum Gen 2007;80;805-810.
24. Graadt van Roggen JF, Hogendoorn PCW, Fletcher CDM. Myxoid tumors of soft tissue. Histopathology 1999;35 :291-312.
25. Fadare O. Myxoid epithelioid sarcoma: Clinicopathologic analysis of 2 cases. Int J Surg Pathol 2009; 17:147-152.
26. Quante M, Patel NK, Hill S, et al. Epithelioid hemangioendothelioma presenting in the skin: A clinico-patholologic study of eight cases. Am J Dermatopathol 1998;20: 541-546.
27. Weiss SW, Ishak KG, Dail DH, Sweet DE, Enzinger FM. Epithelioid hemangioendothelioma and related lesions. Semin Diagn Pathol 1986;3 :259-287.
28. Mentzel T, Beham A, Calonje E, Katenkamp D, Fletcher CDM. Epithelioid hemangioendothelioma of skin and soft tissues : clinicopathologic and imuunohistochemical study of 30 cases. Am J Surg Pathol 1997; 21:363-374.
29. Billings SD, Folpe AL, Weiss SW. Epithelioid sarcoma like hemangioendothelioma. Am J Surg Pathol 2003;27:48-57.
30. Lucas DR, Fletcher CDM, Adsay NV, Zalupski MM. High-grade extraskeletal myxoid chondrosarcoma: a high-grade epithelioid malignancy. Histopathology 1999;35:201-208.
31. Kohashi K, Oda Y, Yamamoto H et al. SMARCB1/INI1 protein expression in round cell soft tissue sarcomas associated with chromosomal translocations involving EWS: A special reference to SMARCB1/INI1 negative variant extraskeletal myxoid chondrsarcoma. Am J Surg Pathol 2008;32:1168-1174.
32. Oliveira AM, Sebo TJ, McGrory JE, Gaffey TA, Rock MG, Nascimento AG. Extraskeletal myxoid chondrosarcoma: a clinicopathologic, immunohistochemical, and ploidy analysis of 23 cases. Mod Pathol 2000;13:900-908.
33. Sciot R, Dal Cin P, Fletcher C, et al. T(9;22) (q22-31;q11-12) is a consistent marker of extraskeletal myxoid chondrsarcoma: evaluation of three cases. Mod Pathol 1995;8:765-768.
34. Hisaoka M, Ishida T, Imamura T, Hashimoto H. TFG is a novel fusion partner of NOR1 in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer 2004;40:325-328.
35. Nascimento AF, Bertoni F, Fletcher CDM. Epithelioid variant of myxofbrosarcoma: expanding the clinicomorphologic spectrum of myxofibrosarcoma in a series of 17 cases. Am J Surg Pathol 2007;31:99-105.

Chapter 6

Presence of *C11orf95-MKL2* fusion is a consistent finding in chondroid lipomas: a study of 8 cases

Histopathology 2013 May;62(6):925-30.

Abstract

Chondroid lipomas are benign adipose tissue tumors. Their rarity and peculiar morphology can lead to misinterpretation, especially in small biopsies. Based on a recurrent translocation t(11;16)(q13;p13), the *C11orf95-MKL2* fusion gene has been found in a few cases. Therefore, it seemed appropriate to look for this fusion gene in a larger cohort.

We describe eight further cases from four females and four males with an age range of 21-81 years (median, 49 years). The tumors were situated in the lower arm (three), lower leg (two), thigh (one), back (one), and head (one); seven lesions were deep-seated and one was located subcutaneously. Sizes ranged from 3 to 12 cm (median, 6.3 cm). All patients were treated by simple excision, and follow-up, available for six patients (range 2 months – 12 years; median 15 months), demonstrated recurrence in one case. Histologically, the circumscribed and lobulated tumors showed a variable composition of adipocytes, lipoblasts, hibernoma-like cells and chondroblast-like cells embedded in a chondroid matrix.

Immunohistochemistry, performed in four cases, revealed positivity for S-100 and pancytokeratin in two of three neoplasms stained for each marker. A *C11orf95-MKL2* fusion gene was shown by RT-PCR analysis in seven of the eight cases.

Molecular analysis can be used to support the diagnosis of chondroid lipoma, especially in small samples. This may be helpful in planning treatment when the differential diagnosis includes malignant lesions.

Introduction

Chondroid lipomas are rare, often deep-seated, benign lipogenic tumors occurring mainly in the extremities and limb girdles of adults. A female preponderance has been reported.[1]

The histomorphological hallmark is an admixture of adipocytes, lipoblasts, hibernoma-like cells and chondroblast-like cells embedded in a myxohyaline chondroid matrix.[1]

A characteristic chromosomal abnormality has been demonstrated and recently, the corresponding fusion gene was detected in three of three cases.[2-5]

The use of minimally invasive biopsies has become increasingly common, and molecular genetic testing can serve as a useful diagnostic adjunct. Therefore, it seemed appropriate to look for this fusion gene in a larger cohort.

Material and methods

The cases were retrieved from the authors' (referral) files, and clinical details and follow-up were obtained from the referring physicians. Details of two of the cases have been published previously.[6,7] Tissue was fixed in 4% buffered formalin, routinely processed and embedded in paraffin; 2-4 µm thick sections were stained with hematoxylin and eosin and immunohistochemically by the labelled streptavidinbiotin technique using commercially available antibodies as listed in Table 1. Appropriate positive and negative controls were used throughout.

Translocation-specific RT-PCR

cDNA synthesis was performed in a 24 ul reaction mixture containing 1 ug of RNA, 1 ug of random hexamers (Promega, Madison, WI, USA) and 20 nmol dNTPs (Invitrogen, Carlsbad, CA, USA) and heated at 65 °C for 5 min. Next 2 ul of RNasin (Promega), 8 ul of 5x first strand buffer (Invitrogen), 4 ul of 0.1 M dithiothreitol (Invitrogen) and 2 ul of Superscript II reverse transcriptase (Invitrogen) were added and the samples were heated as follows: 10 min at 20 °C, 60 min at 42 °C and 3 min at 95 °C. cDNA was stored at -20 °C.

Translocation-specific *C11orf95-MKL2* fusion products, as described by Huang et al[5] were detected using primers targeting *C11orf95* (5' GAGTACCTGATGGACTACGAC 3') and *MKL2* (5' GACCCCTTAACTTCAGTTCTG 3').

PCR was carried out in duplicate in a PTC 200 Thermal Cycler (MJ Research, Waltham, MA, USA) starting with 10 min at 95 °C and followed by 38 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72°C for 60 s, with a final extension at 72°C for 7 min and then cooling down for 5 min at 20°C. PCR products were analyzed by 2% agarose gel electrophoresis.

Table 1 Details of used immunohistochemical antibodies

Antibody	Clone	Dilution	Source
ASMA	1A4	1:500	DAKO, Glostrup, Denmark
EMA	Mc5	1:400	BioGenex, San Ramon, USA
Pan-cytokeratin	MNF116	1:500	DAKO, Glostrup, Denmark
Pan-cytokeratin	AE1/3	1:50	DAKO, Glostrup, Denmark
S-100	polyclonal	1:2000	DAKO, Glostrup, Denmark

Results

Clinical data are presented in Table 2. The eight lesions were from four females and four males, with an age range of 21-81 years (median, 49 years). The tumors were situated in the lower arm (three), lower leg (two), thigh (one), back (one), and head (one). Seven neoplasms were deep seated and one was located subcutaneously. The size ranged from 3 to 12 cm (median, 6.3 cm). Follow-up available for 6 patients (2 months – 12 years; median interval, 15 months) showed a recurrence in one case (case 5) after 5 years. All other patients were cured by simple excision. The tumors appeared grossly well circumscribed and encapsulated, with a lobulated and yellowish cut surface (Figure 1).

Microscopy confirmed the lesions to be well circumscribed and encapsulated. In several cases the presence of septa resulted in a multinodular appearance. The adipocytic component ranged from 10% to 70% and was admixed with hibernoma-like cells, lipoblasts and chondroblast-like cells. The eosinophilic cells showed arrangement in sheets, nests and cords. The nuclei varied in shape from oval to reniform and were placed centrally or peripherally, with some variation in nuclear size in four cases. Classical lipoblasts had indented nuclei. Prominent nucleoli and mitotic figures were not seen. The myxohyaline matrix was prominent in two cases. A striking feature in all cases was a prominent vasculature with thick- and thin-walled vessels. Some of the vessels were hyalinized (Figure 2).

Immunohistochemistry was performed on four tumors. Two of three were positive for S-100. One (one of one) was positive with the pan-cytokeratin antibody MNF 116 (Figure 3) and another (one of two) with pan-cytokeratin AE1/3. Immunohistochemistry for epithelial membrane antigen (EMA) and smooth muscle actin was performed in one case each, with negative results (Table 3).

By RT-PCR, we found *C11orf95-MKL2* fusion in seven of eight cases (Table 3). All seven cases harboured a translocation resulting in a fusion between exon 5 of *C11orf95* and exon 9 of *MKL2.* Six cases had the same fusion-product as described by

Figure 1 Case 1: grossly, the tumour looks like a usual lipoma (with a multilobular pattern)

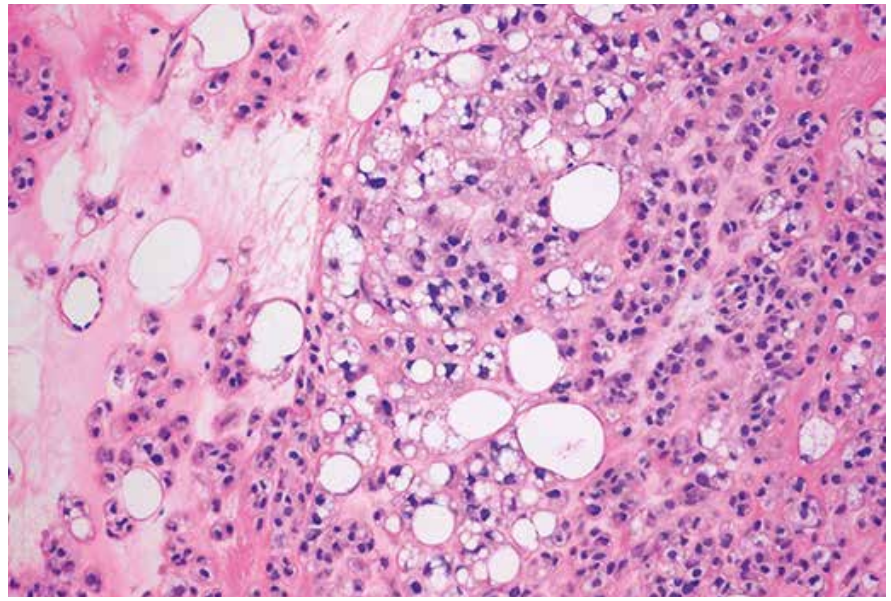

Figure 2 The tumours show an admixture of adipocytes, lipoblasts, hibernoma-like cells and chondroblast-like cells set in a myxohyaline, chondroid matrix

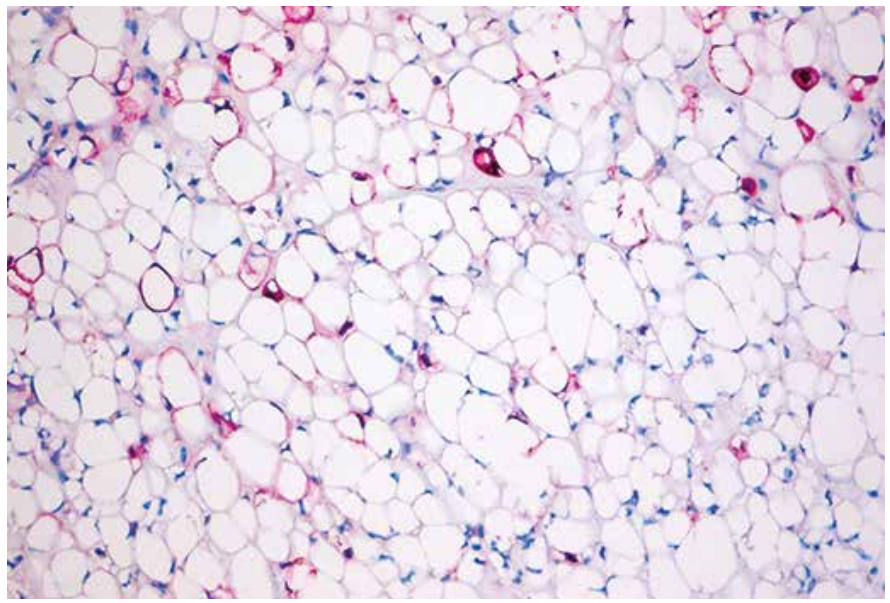

Figure 3 Pan-keratin may be expressed in this tumor type (Case 6)

Table 2 Clinical data

Case	Age/Sex	Localization	Size (cm)	Follow-up
1	41 y/f	lower arm, deep	4.5	2 mos, NED
2	21 y/f	lower leg, deep	4.5	17 mos, NED
3	38 y/f	thigh, deep	12	12 ys, NED
4	56 y/m	back deep	3	7 mos, NED
5	57 y/m	lower arm, sc	8	12 ys, 1 rec after 5ys
6	34 y/m	lower arm, deep	NA	12 mos, NED
7	81 y/m	infratemporal fossa, deep	NA	NA
8	69 y/f	calf, deep	11.4	NA

Y, years; f, female; m, male; NA, information not available; sc, subcutaneous; rec, recurrence; mos, months; ys, years; NED, no evidence of disease

Huang et al.[5] In one case (Case 1), an alternative breakpoint in *C11orf95* was detected (Figure 4). The PCR product was 39 base-pairs smaller.

Two hibernomas, of a 25 year old female and a 59 year old male, both located in the upper leg were used as controls and were negative for the gene fusion.

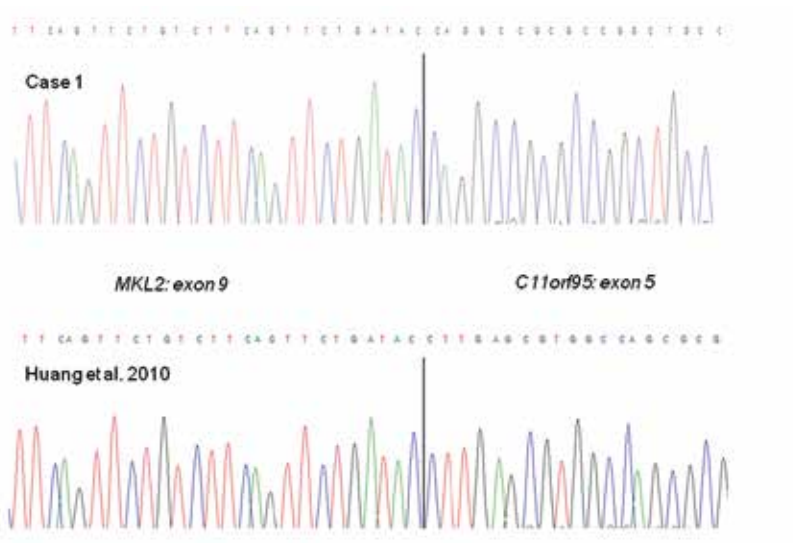

Figure 4 The sequence of RT-PCR product shows a fusion between exon 5 of *C11orf95* and exon 9 of *MKL2* in the chimeric transcript in 7/8 cases

Table 3 Immunohistochemical and molecular data

Case	S-100	Pan-CK	EMA	ASMA	*C11orf95-MKL2* (exon 5 - exon 9)
1	-	-	nd	-	+
2	+	nd	nd	nd	+
3	nd	nd	nd	nd	+
4	nd	nd	nd	nd	+
5	nd	nd	nd	nd	+
6	nd	+	nd	nd	+
7	+	+	-	nd	-
8	nd	nd	nd	nd	+

+, positive; -, negative; nd, not done

Discussion

Adipocytic tumors represent the largest group of soft tissue tumors in general.[1,6] They have been studied extensively by cytogenetic analysis, and the first reported consistent karyotypic abnormality has been discovered in lipomas.[8]

Recurrent involvement of 11q13 was found in the karyotypic alterations of lipoma, hibernoma and chondroid lipoma.[2-5,9-13] Chondroid lipoma shows a consistent t(11;16)(q13;p13) translocation resulting in the fusion oncogene *C11orf95-MKL2*.[2-5] Six of our cases harboured the previously described breakpoint regions[5] and, in one case, an alternative breakpoint in *C11orf95* was detected generating a fusion-product that involved the same exons. Interestingly, the structure of the genes as illustrated by Huang et al.[5] differs from that of the Reference Sequence (RefSeq) database genes, and it is therefore difficult to annotate these fusion-products accurately. The MKL2 protein is known to be involved in chromatin remodeling and transcription. The function of C11orf95 is as yet unknown.[5]

As indicated by our series, *C11orf95-MKL2* is a consistent finding in chondroid lipomas and can be of diagnostically value, especially in small samples, because of the therapeutic consequences when malignant lesions are included in the differential diagnoses.

When Meis and Enzinger first described chondroid lipoma[1] they underscored the resemblance to myxoid liposaroma (MLS) and extraskeletal myxoid chondrosarcoma (EMC). Both of these entities share the myxohyaline chondroid matrix, but neither has the prominent vasculature varying from thick-walled to thin-walled and cavernous blood vessels. The delicate plexiform capillaries, characteristic for MLS, are absent in chondroid lipoma, as are the typical primitive cells.[1] Cords and nests of small chondroblast-like eosinophilic cells are an overlapping feature with EMC. Although some vacuolated cells can occur in EMC, there is no adipocytic/lipoblastic component. However, extensive myxoid change can obscure the latter.[1] S-100 immunohistochemistry can be positive in all three tumor types and is therefore of little value. Both sarcomas possess a specific genetic abnormality with a fusion gene involving *NR4A3* in EMC and *DDIT3* in MLS.[12]

Myoepithelioma, especially with 'parachordoma'- like appearance, enters the differential diagnosis.[1] In addition to the architecture and cytomorphology (nest- and cord-like arrangement of clear to eosinophilic cells), the myxohyaline chondroid matrix and positivity for S-100 and keratin can cause confusion with chondroid lipomas. In this context, the heterogeneous composition of myoepithelial cells, sometimes with tubuloductal structures, stronger expression of keratins and/or EMA and the absence of lipoblasts, are discriminating signs. Conversly, adipocytes can be present in myoepithelial tumors.[14-17] There is a distinct genetic background with *EWSR1* or *PLAG1* rearrangements in a subset of cases.[18,19]

Although occurring very infrequently, chordoma periphericum is one of the differential diagnoses. The histomorphology is similar to axial chordomas, with nuclear atypia of the clear to eosinophilic physaliferous cells. A lipogenic component does not exist. Brachyury is the key immunohistochemical marker providing evidence for notochordal differentiation.[20]

Hibernoma consists of brown fat cells with granular, multivacuolated cytoplasm and mature adipocytes in varying amounts. Although, myxoid changes can occur in all lipomatous tumors, a prominent chondromyxoid matrix is not typical for hibernoma, and chondroblast-like cells are absent.[12,21,22] Breakpoints in 11q13.5 near *GARP* and deletions of various genes of 11q13 were detected.[8,10] But *CIIorf95* is not involved, as demonstrated by FISH[5] and, in our two cases, by RT-PCR.

Lipoblastoma is an adipose lesion of infancy and early childhood with nearly 90% occurring before 3 years of age.[22] It has been rarely reported in adolescents and young adults.[22,23] These lobulated tumors are composed of fat cells with varying degrees of differentiation, including primitive cells or prelipoblasts, lipoblasts and adipocytes. Hibernoma-like cells can occur in contrast to chondroid cells. Myxoid stroma may be prominent in these lesions and the fibrous septa contain numerous capillaries.[6,22-24] Genetically, most lipoblastomas show a rearrangement of *PLAG1* located on chromosome 8q12 and/or possess extra copies of the same chromosome.[22,23] Alternatively, *HMGA2* can be rearranged.[25]

Metaplastic cartilage has been described in a variety of lipogenic tumors, such as chondrolipoma, lipoblastoma, myxoid liposarcoma and dedifferentiated liposarcoma.[1,6,24,26] This supports the notion of overlapping pathways in lipo- and chondrogenesis;[5,27] therefore, it is not surprising that lipo- and chondrogenic features are also discussed in chondroid lipomas.[5,26]

Recurrent chromosome rearrangements, in particular translocations, are strongly associated with distinct tumor entities.[28] However, overlap in different tumor types is well known.[12,29] There is compelling evidence that they represent an initial event in oncogenesis.[28]

The treatment of chondroid lipoma is simple excision and recurrence has been rarely reported.[30] In our series, only one patient had a recurrence, after 5 years. This corroborates the benign nature of the lesion.[1,6]

In summary, chondroid lipoma is a very rare, often deep-seated benign lipogenic tumor with chondroid features and a consistent molecular abnormality: the detection of *CIIorf95-MKL2* can provide diagnostic confirmation.

References

1. Meis JM, Enzinger FM**.** Chondroid lipoma. A unique tumor simulating liposarcoma and myxoid chondrosarcoma. Am J Surg Pathol 1993;17:1103-1112.
2. Gisselson D, Domanski HA, Höglund M, et al. Unique cytological features and chromosome aberrations in chondroid lipoma: a case report based on fine-needle aspiration cytology, histopathology, electron microscopy, chromosome banding, and molecular cytogenetics. Am J Surg Pathol 1999;23:1300-1304.
3. Thomson TA, Horsman D, Bainbridge TC. Cytogenetic and cytologic features of chondroid lipoma of soft tissue. Mod Pathol 1999;12:88-91.
4. Ballaux F, Debiec-Rychter M, De Wever I, Sciot R. Chondroid lipoma is characterized by t(11;16)(q13;p12-13). Virchows Arch 2004;444:208-210.
5. Huang D, Sumegi J, Dal Cin P, et al. C11orf95 is the resulting fusion oncogene of t(11;16)(q13;p13) in chondroid lipoma. Genes Chromosomes Cancer 2010;49:810-818.
6. Mentzel T, Remmler K, Katenkamp D. Chondroides Lipom. Klinisch-pathologische, immunhistochemische und ultrastrukturelle Analyse von 6 Fällen einer distinkten Entität im Spektrum der Lipome. Pathologe 1999;20:330-334.
7. De Vreeze RS, van Coevorden F, Boerrigter L, et al. Delineation of chondroid lipoma: an immunohistochemical and molecular biological analysis. Sarcoma 2011;2011: 638403.
8. Nishio J. Contributions of cytogenetics and molecular cytogenetics to the diagnosis of adipocytic tumors. J Biomed Biotechnol 2011;2011;524067.
9. Sreekantaiah C, Leong SPL, Karakousis CP, et al. Cytogenetic profile of 109 Lipomas. Cancer Res. 1991;51: 422-433.
10. Maire G, Forus A, Foa C, et al. 11q13 alterations in two cases of hibernoma: large heterozygous deletions and rearrangement breakpoints near GARP in 11q13.5. Genes Chromosomes Cancer 2003;37:389-395.
11. Rusconi D, Valtorta E, Rodeschini O, et al. Combined characterization of the pituitary adenoma and a subcutaneous lipoma in a MEN1 patient with a whole gene deletion. Cancer Genetics 2011;204:309-315.
12. Fletcher CDM, Unni K, Mertens F, eds. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft tissue and Bone. IARC press: Lyon, France; 2002, pp 33,40,194,211,213.
13. Mertens F, Rydholm A, Brosjö H, et al. Hibernomas are characterized by rearrangements of chromosome bands 11q13-21. Int J Cancer 1994;58:503-505.
14. Kilpatrick SE, Hitchcock MG, Kraus MD, et al. Mixed tumors and myoepitheliomas of soft tissue: A clinicopathologic study of 19 cases with a unifying concept. Am J Surg Pathol 1997;21:13-22.
15. Folpe AL, Agoff SN, Willis J, Weiss SW. Parachordoma is immunohistochemically and cytogenetically distinct from axial chordoma and extraskeletal myxoid chondrosarcoma. Am J Surg Pathol 1999;23:1059-1067.
16. Hornick JL, Fletcher CDM. Myoepithelial tumors of soft tissue. A clinicopathologic and immunohistochemical study of 101 cases with evaluation of prognostic parameters. Am J Surg Pathol 2003;27:1183-1196.
17. Gleason BC, Hornick JL. Myoepithelial tumours of skin and soft tissue: an update. Diagn Histopathol 2008;14:552-562.
18. Antonescu CR, Zhang L, Chang NE, et al. *EWSR1-POU5F1* fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone and visceral lesions showing common involvement of EWSR1 gene rearrangement. Genes Chromosomes Cancer 2010;49:1114-1124.
19. Bahrami A, Dalton JD, Krane JF, Fletcher CDM. A subset of cutaneous and soft tissue mixed tumors are genetically linked to their salivary gland counterpart. Genes Chromosomes Cancer 2012;51:140-148.
20. Tirabosco R, Mangham DC, Rosenberg AE, et al. Brachyury expression in extra-axial skeletal and soft tissue chordomas: a marker that distinguishes chordoma from mixed tumor/myoepithelioma/parachordoma in soft tissue. Am J Surg Pathol 2008;32:572-580.
21. Furlong MA, Fanburg-Smith JC, Miettinen M. The morphologic spectrum of hibernoma. A clinicopathologic study of 170 cases. Am J Surg Pathol 2001;25:809-814.
22. Coffin CM, Alaggio R. Adipose and myxoid tumors of childhood and adolescence. Pediatr Dev Pathol 2012;15:239-254.
23. de Saint Aubain Somerhausen N, Coindre JM, Debiec-Rychter M, Delplace J, Sciot R. Lipoblastoma in adolescents and young adults: report of six cases with FISH analysis. Histopathology 2008;52:294-298.
24. Chung EB, Enzinger FM. Benign lipoblastomatosis. An analysis of 35 cases. Cancer 1973;32:482-492.
25. Pedeutour F, Deville A, Steyaert H, et al. Rearrangement of HMGA2 in a case of infantile lipoblastoma without Plag1 alteration. Pediatr Blood Cancer 2012;58:798-800.
26. Kindblom LG, Meis-Kindblom JM. Chondroid lipoma: an ultrastructural and imunohistochemical analysis with further observations regarding its differentiation. Hum Pathol 1995;26:706-715.
27. Kubo T, Matsui Y, Goto T, et al. Overexpression of HMGA2-LPP fusion transcripts promotes expression of the a-2 type XI collagen gene. Biochem Biophys Res Commun 2006;340:476-481.
28. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer 2007;7:233-245.
29. Thway K, Fisher C. Tumors with EWSR1-CREB1 and EWSR1-ATF1 fusions: The current status. Am J Surg Pathol 2012;36:e1-e11.
30. Miettinen M ed. Modern soft tissue pathology. Tumors and non-neoplastic conditions. Cambridge University Press 2010, p 414.

Chapter 7

Cellular angiofibroma: analysis of 25 cases emphasizing its relationship to spindle cell lipoma and mammary-type myofibroblastoma

Mod Pathol 2011 Jan;24(1):82-9.

Abstract

Cellular angiofibroma represents a rare benign mesenchymal tumor, occurring mainly in the superficial soft tissue of the genital region. The involvement of 13q14 in some cases confirmed the morphologic suggested link with spindle cell lipoma and mammary-type myofibroblastoma. We analysed the clinicopathologic and immunohistochemical features of 25 cases, and performed in a number of cases additional molecular studies. There were 17 female and 8 male patients (age ranged from 27 to 83 years); females tended to be younger. A marked predilection for the vulva (n=13) was observed, and neoplasms in males were predominantly located in the inguinal region (n=4), and one case each in the scrotum, perianal, the knee, and the upper eyelid. The tumors arose most commonly in the superficial soft tissue and were well circumscribed in all but two cases. The tumor size ranged from 1 cm to 9 cm. All lesions were composed of spindle-shaped cells associated with numerous small- to medium-sized blood vessels, however a broad morphologic variation with foci of lipogenic differentiation in 9 cases and sarcomatous transformation in one case was found. By immunohistochemistry, 11 out of 22 cases expressed CD34. A focal reaction for α-smooth muscle actin was observed in 9 out of 22 cases, and two cases each stained weak and focally positive for epithelial membrane antigen and CD99. In all 7 cases tested, a monoallelic deletion of *RB1* was detected by FISH-analysis. Follow-up, available in 14 patients, showed neither local recurrence nor metastasis. In conclusion, we affirm the link between cellular angiofibroma, spindle cell lipoma and mammary-type myofibroblastoma, showing a spectrum of one entity with morphological variations dependent on anatomic location.

Introduction

Cellular angiofibroma, first described in 1997 by Nucci et al, is an uncommon benign mesenchymal tumor occurring mainly in the genital region of both genders.[1-3] Extragenital localisations are also known; however, most of all lesions are assigned to the pelvic area.[1-7] They are commonly well-circumscribed, localized in the superficial soft tissue and characterized by bland spindle-shaped cells arranged without any pattern in a stroma with wispy collagen and numerous small- to medium-sized thick walled vessels.[1-3]

More recently, atypical features and sarcomatous transformation of cellular angiofibroma have been reported. Whereas in some cases, areas of pleomorphic spindle cells were examined, others contained a well-differentiated liposarcoma or a pleomorphic liposarcoma component, but in neither of these patients a poor outcome was present.[3,6,8]

The histomorphological and, to a lesser extent, immunohistochemical overlap of cellular angiofibroma with spindle cell lipoma and mammary-type myofibroblastoma suggested a possible link among them.[1-3,9-11] Subsequently, a monoallelic loss of *RB1* and *FOXO1*, located on 13q14 was found in some of these tumor types supporting the notion that they may belong to a spectrum.[12-16]

We report on 25 additional cases of CAF to widen the clinicopathologic features with an additional morphologically sarcomatous transformed case, and underpin the genetic relationship with mammary-type myofibroblastoma and spindle cell lipoma.

Materials and methods

The cases were retrieved from the authors' referral files, and clinical details and follow-up were obtained from the referring pathologists (see acknowledgement). In all cases, the tissue was fixed in 4% buffered formalin, routinely processed, and embedded in paraffin; 2-4 µm thick sections were stained with hematoxylin and eosin and immunohistochemically by the labelled Streptavidin Biotin technique using commercially available antibodies listed in Table 1. Antigen retrieval was performed for all of them. Appropriate positive and negative controls were used throughout.

FISH-analysis for the detection of *RB1*, located on 13q14.2, was performed in seven cases with a direct spectrum orange labelled probe (Abbott, Bergisch Gladbach, Germany) on 3 µm sections of formalin-fixed, paraffin-embedded tissue after baking at 65°C for 16 hours, deparaffinization with xylene and rehydration with ethanol. All tissue sections were pre-treated with a 30% solution of Oncor pre-treatment solution and digested with Proteinase K following the instructions of the suppliers (Q-Biogene, Heidelberg, Germany). Digestion times were optimized on a case-by-case basis. After

Table 1 Details of used immunohistochemical antibodies

Antibody	Clone	Dilution	Source
ASMA	1A4	1:500	DAKO
Desmin	D33	1:200	DAKO
CD117	polyclonal	1:100	DAKO
EMA	Mc5	1:400	BioGenex, San Ramon, USA
CD34	HPCA-1	1:100	BD Biosciences, San Jose, USA
Pancytokeratin	MNF116	1:500	DAKO
S-100 protein	polyclonal	1:2000	DAKO
h-Caldesmon	h-CD	1:200	DAKO
CD31	JC70A	1:100	DAKO
CD99	12E7	1:500	DAKO

a second rehydration step, the probes were applied to the sections and the covered slides were sealed with rubber cement, heat-denatured and hybridised at 37°C for 16 hours. One positive (spindle cell lipoma) and one negative control (normal tissue) were included in each FISH-series.

After stringent washing with 50 % formamide in 2xSSC and treating with FITC anti-DIG in case of the indirectly labelled probes, the sections were counterstained with DAPI II in mounting medium (125 ng/ml, Abbott, Bergisch Gladbach, Germany) and visualized under a Zeiss Axioplan 2 microscope using a HBO103 lamp and the appropriate filters for three fluorescence dyes.

Results

Clinical Findings

Clinical details are summarized in Table 2. Briefly, the neoplasms arose in 17 female and 8 male patients. The age range was 27-83 years (mean: 52 years; median: 50 years). Women tended to be younger (age range 27 – 63 years; mean: 47 years; median: 47 years) than men (age range 32 – 83 years; mean: 63 years, median: 67 years). Tumors arising in female patients were predominantly seen in the vulva (n=13). Four of them were assigned to the labia majora and one to the clitoris. One case each was located in the vaginal introitus, vaginal fornix, perineum, and the knee. Lesions in male patients were seen in the inguinal region (n=4), the scrotum (n=1), in the perianal region (n=1), the knee (n=1) and the upper eyelid (n=1). All patients underwent simple

Table 2 Clinical data of 25 cases of cellular angiofibroma

Case	Sex/Age	Site	Size (cm)	Treatment	Follow-Up (mos)
1	m/48	scrotum	9	R0	110, NER
2	f/41	perineal	3	R0	NA
3	m/75	groin	6.5	R0	48, NER
4	f/39	vaginal introitus	1	R1	75, NER
5	f/50	vulva	3	R1	55, NER
6	f/51	labium majus	2.7	ME	66, NER
7	f/44	labium majus	2.3	R0	NA
8	f/50	vulva	4	R2	NA
9	f/63	knee lateral	4	R0	45, NER
10	f/48	vulva	8.5	R0	NA
11	f/42	vulva	2.2	R0	NA
12	f/63	clitoris	2.5	R1	38, NER
13	f/27	labium majus	8	ME	NA
14	f/42	vulva	1.7	R0	30, NER
15	f/46	labium majus	3	ME	NA
16	m/83	inguinal canal	2	R0	NA
17	f/55	vulva	2.3	R0	12, NER
18	m/77	inguinal canal	4.5	R0	NA
19	m/56	inguinal canal	3	R0	6, NER
20	m/77	knee	5.1	R0	11, NER
21	f/57	vulva	4.5	RX	6, NER
22	f/47	vulva	1.5	R1	NA
23	m/32	upper eyelid	1.2	R1	6, NER
24	m/59	perianal	8	R1	7, NER
25	f/39	vaginal fornix	9	ME	recently

m, male; f, female; R0, complete excision; ME, marginal excision; R1, local excision with histological positive margins; R2, local excision with macroscopically positive margins; RX, local excision, margins not known
NA, information not available; NER, no evidence of recurrence

local excision. Complete excision was reached in 13 cases and re-excision with subsequent tumor free margins was performed in one case (Case 2). Marginal excision was verified in four cases and positive margins were reported in seven cases. The resection status was not known for Case 21.

Follow-up, available for 14 patients, ranged from 6 to 110 months (mean: 37 months; median: 34 months). It was unremarkable for all patients. Case 5 most likely represents a relapse of an 11 years earlier excised tumor.

Given clinical diagnoses were (Bartholin) cyst, polyp, myoma, atheroma, pilomatricoma or lacrimal gland carcinoma. The last two diagnoses were assigned to the tumor in the upper eyelid.

Pathologic Findings

Grossly, the neoplasms were described as white or yellowish nodules, mostly solid and partly gelatinous or cystic in appearance. Three tumors were recorded as polypoid lesions (Case 11, 12, 25). The size ranged from 1cm to 9 cm (mean: 4.1 cm). Tumors in women inclined to be smaller (range: 1 cm – 9 cm; mean: 3.7 cm) than in men (range: 1.2 cm – 9 cm; mean: 4.9 cm).

Histologically, most lesions were found in superficial soft tissue (n=21) and all but two were well circumscribed. Nine tumors were at least partly encapsulated. An infiltrative growth was seen in Case 5 and 21. The first was located in deep soft tissue while the second showed features of sarcomatous transformation (see below). Three vulvar and one vaginal lesion were superficially located and showed an exophytic growth, one of them (Case 10) was ulcerated. In 11 cases, a multinodular growth with incomplete fibrous septa was noted. The spindle-shaped tumor cells were arranged haphazardly and showed organisation partly in short fascicles in four cases. They had bland, oval to fusiform and sometimes tapering nuclei. The cytoplasm was ill-defined, pale eosinophilic and had, if recognizable, bipolar processes (Figure 1). Nuclear grooves and intranuclear inclusions were commonly observed. In three cases (Cases 6, 7, 11), focal mild nuclear atypia with slightly enlarged nuclei was recognizable, and furthermore, in Case 11 some multinucleated cells were detected (Figure 2). Six cases featured mitotic figures, ranging from 1 to 3 mitoses per 10 high-power fields. A transformation from typical areas of CAF into cellular not well-delineated nodules composed of enlarged, highly pleomorphic and multinucleated cells with prominent nuclear atypia and atypical mitoses was present in Case 21 (Figure 3-5). Thus, this part of the tumor has morphological features of malignancy.

All tumors contained wispy collagen; additionally, thicker collagen bundles were focally present in six cases. The vascular component, prominent in all lesions, consisted of small to medium sized vessels with mostly hyalinized walls (Figure 1). A hemangiopericytoma-like vascular pattern was observed in three cases (Cases 16, 19, 21) (Figure 6). Edematous changes of vessels due to chronic inflammation were seen in four cases and vascular thrombotic obliteration was observed in one case. Nine cases (36%) included mature adipocytes, predominantly in peripheral areas, ranging from 1 - 30 % of the analysed tumor area. Pseudovascular spaces filled with proteinaceous fluid were seen in three cases and microcystic changes in four cases

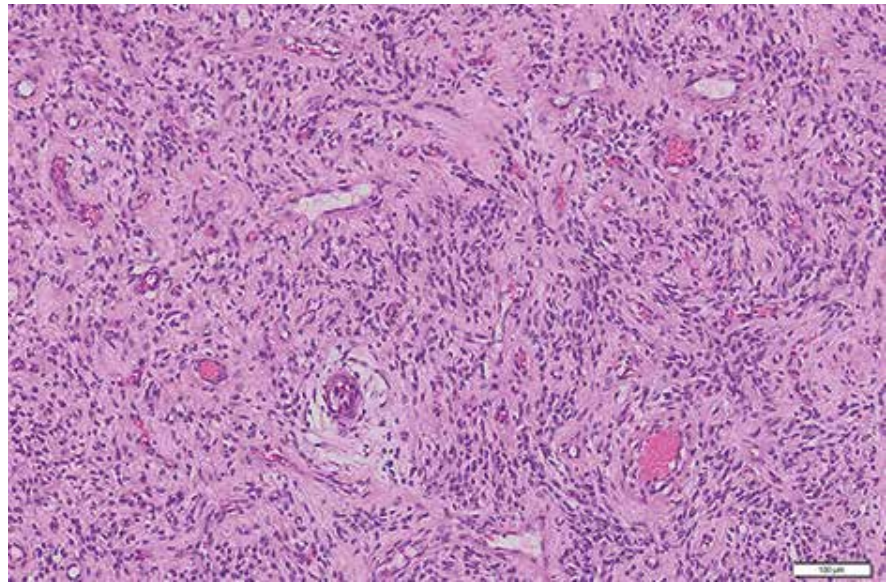

Figure 1 Typical features of CAF showing loosely arranged spindle cell lipoma like cells and a prominent vasculature with thick vessel walls. There is a finely collagenous background (Case 3)

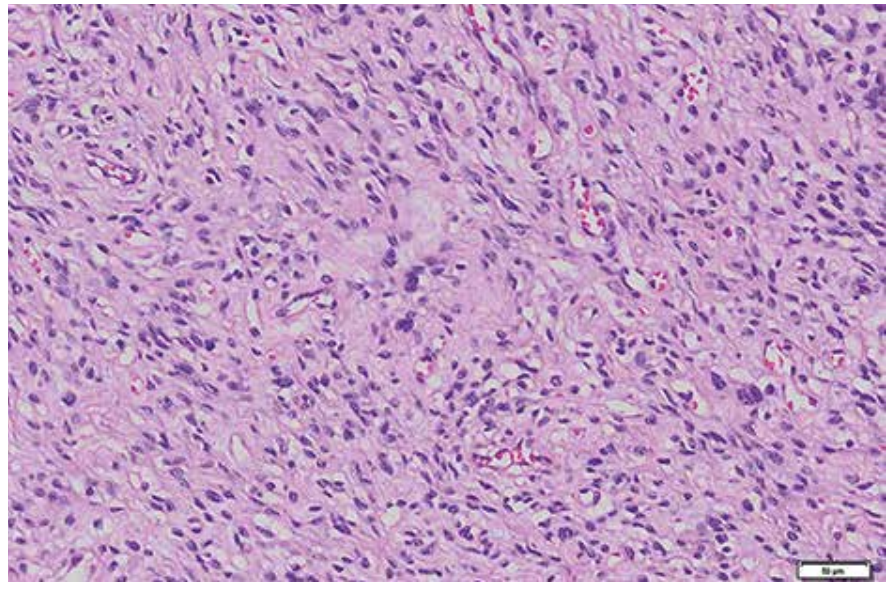

Figure 2 Mild nuclear atypia with slightly enlarged nuclei and multinucleated cells found in Case 11

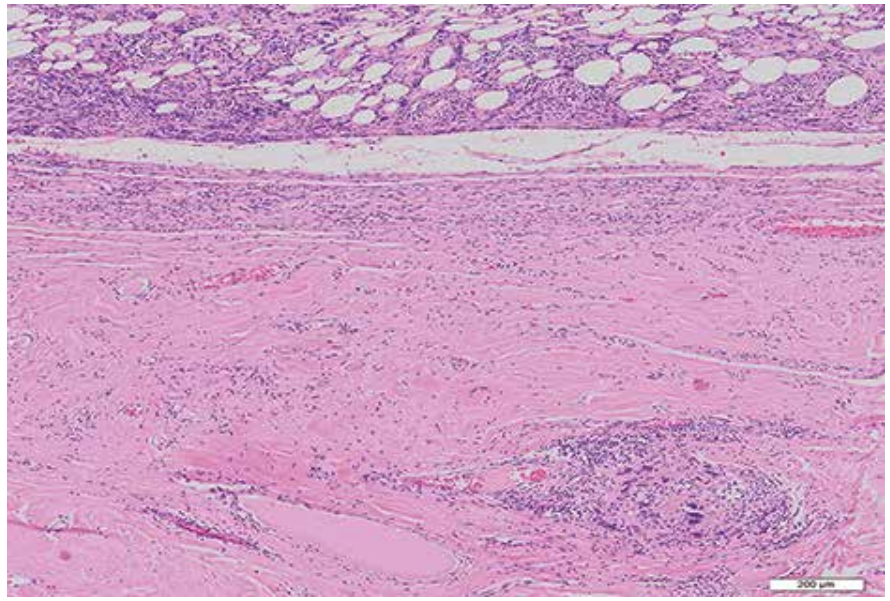

Figure 3 The sarcomatous transformed component in Case 21 was not well circumscribed and included mature adipocytes

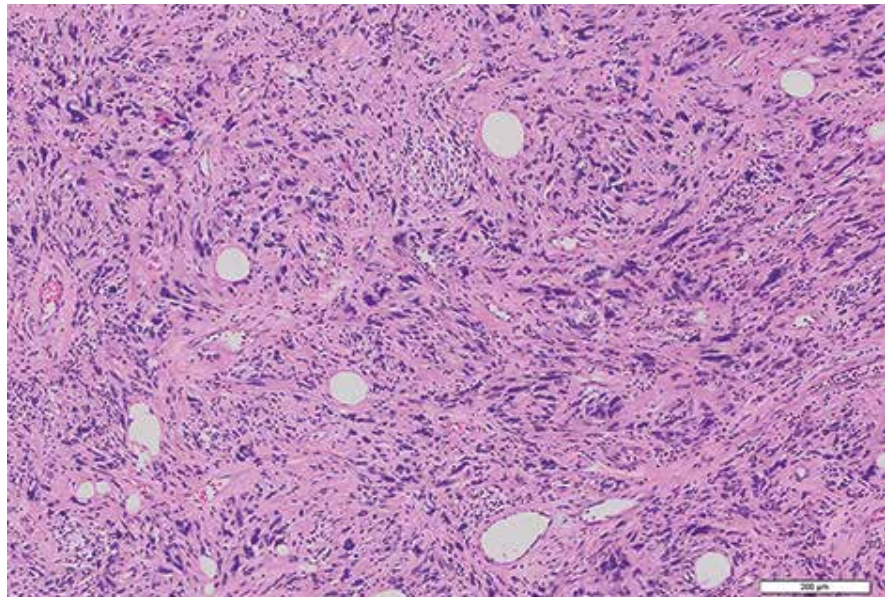

Figure 4 There are pleomorphic nuclei and a typical pattern of CAF with prominent hyalinized vessels (Case 21)

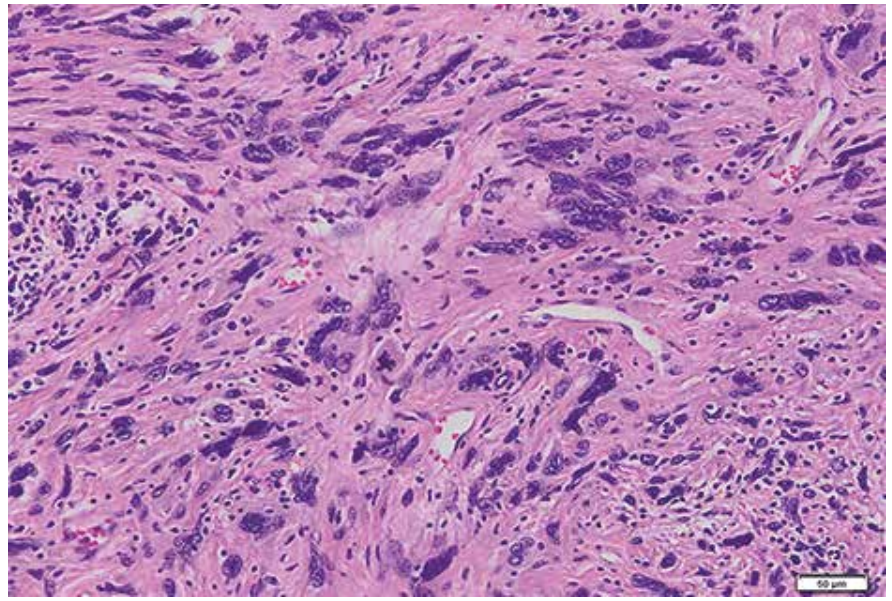

Figure 5 Pleomorphic tumor cells and atypical mitotic figures of the malignant areas of Case 21

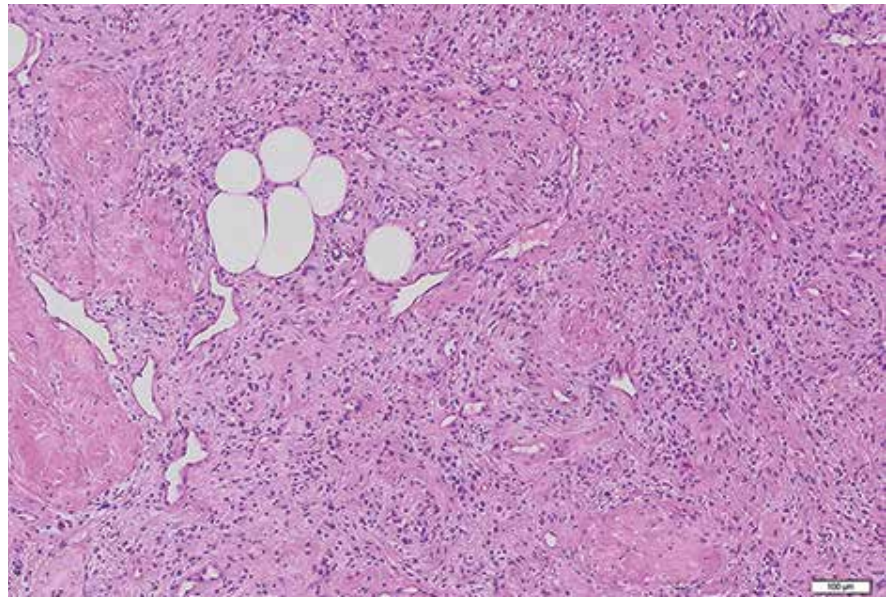

Figure 6 Case 16 shows hemangiopericytoma like vessels and focal inclusion of adipocytes; broad collagen deposits are also seen

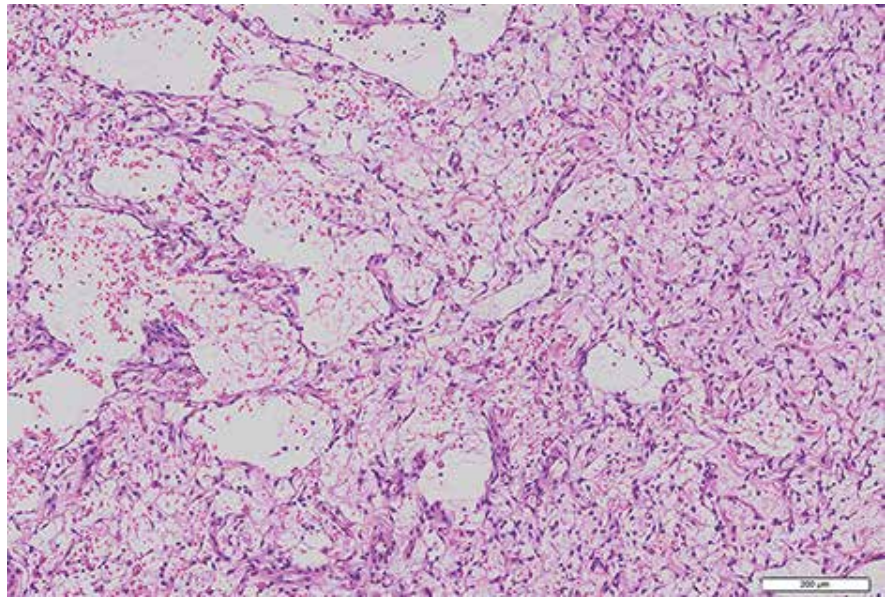

Figure 7 Prominent pseudovascular structures were found in 3 cases, as seen in Case 10

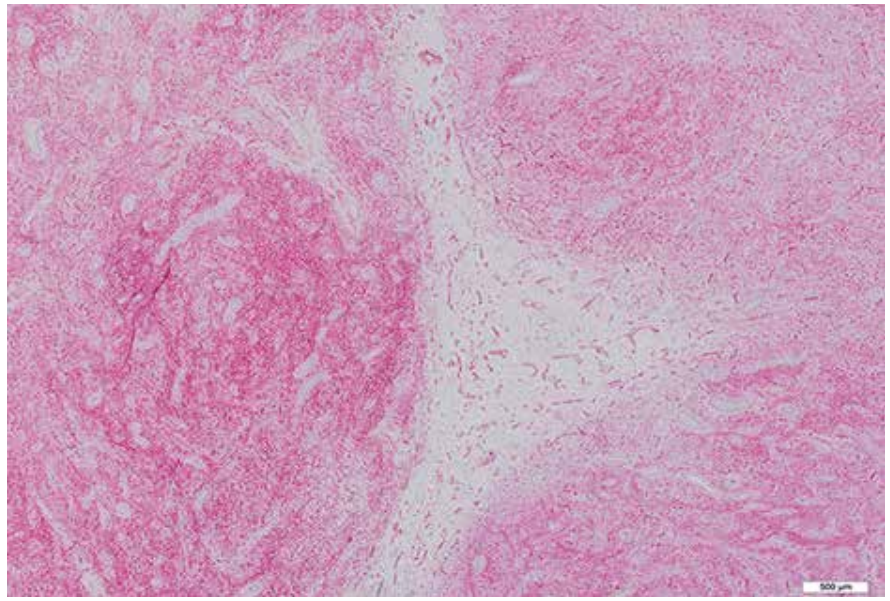

Figure 8 Strong CD34 positivity accented in Case 3 the multinodular architecture

(Figure 7). 17 cases showed at least focally myxoid stromal changes while a hyalinized background was noted in 10 cases. Scattered mast cells were found throughout the lesions, and lymphocytic aggregates, at least focally, were commonly present. Areas of hemorrhage or tumor necrosis were not observed.

Immunohistochemically, 11 out of 22 cases tested expressed CD34 (Figure 8); one of them was only focally positive. α-smooth muscle actin was focally detected in 9 out of 22 cases tested (40,9%), and epithelial membrane antigen (EMA) in two out of six cases. CD99 was positive in two out of four cases. The remaining antibodies (desmin, h-caldesmon, S-100 protein, pancytokeratin, CD31) were negative in all cases tested.

Seven cases analysed by FISH exhibited a monoallelic deletion of the *RB1* in a considerable number of counted nuclei (Table 3, Figure 9).

Table 3 Results of *RB1* FISH Analyses

Case 9	Deletion in 33 out of 63 nuclei counted
Case 10	Deletion in 16 out of 49 nuclei counted
Case 15	Deletion in 27 out of 52 nuclei counted
Case 16	Deletion in 24 out of 51 nuclei counted
Case 17	Deletion in 19 out of 56 nuclei counted
Case 19	Deletion in 42 out of 56 nuclei counted
Case 25	Deletion in 26 out of 54 nuclei counted

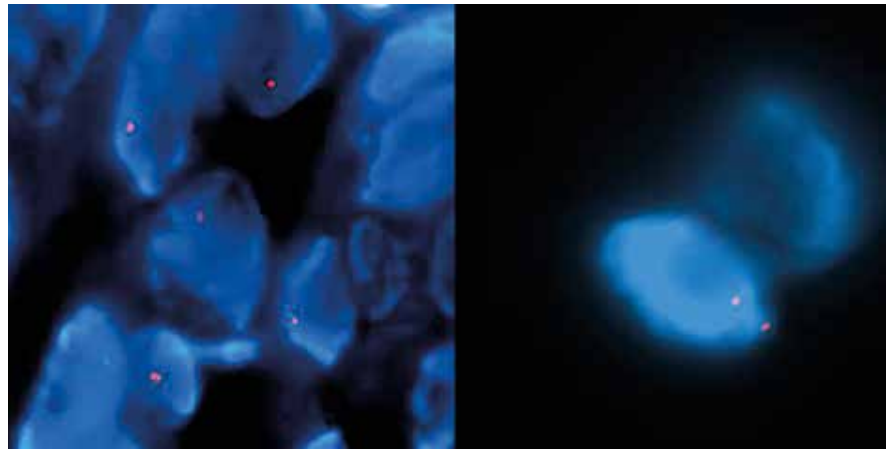

Figure 9 FISH analysis: monoallelic loss of *RB1* was found in all 7 cases tested. A negative control with both alleles of *RB1* is seen on the right

Discussion

Cellular angiofibromas, most commonly described in the genital region of both genders, are rare benign mesenchymal neoplasms characterised by a bland spindle cell component and numerous small- to medium-sized vessels with mural hyalinization. Typically, there is a wispy collagenous, occasionally myxoid stroma. A mature adipocytic component is variably seen.[1-3]

A morphological overlap with spindle cell lipoma and mammary-type myofibroblastoma has been amply described. Whereas a more fascicular pattern has been attributed to the mammary-type myofibroblastoma, a more haphazard arrangement was observed in the other two neoplasms. On the other hand, ropey collagen is a characteristic finding in spindle cell lipoma and mammary-type myofibroblastoma. The striking hyalinized vessels are distinctive for cellular angiofibroma.[1-3,9-11,17,18]

There are few reported lesions in extragenital localisations, including retroperitoneum, pelvic and lumbal region, anus, urethra, trunk, and oral mucosa.[3-7] Our files contained three extragenital tumors located on the knee (n=2) and upper eyelid (n=1). These cases also had classical histological features with obvious hyalinized vessels and, at least focally, finely collagenous background.

Mature adipocytes were found in approximately one third and pseudovascular structures were present in three of our cases. These findings are in line with earlier descriptions.[2,3,19]

Immunohistochemically, expression of CD34 was seen in 50% of the cases tested, whereas none of our cases analyzed showed an expression of desmin in accordance with reports of others.[2,3] In comparison, spindle cell lipoma and mammary type myofibroblastoma are consistent positive for CD34 and the latter shows always coexpression of desmin.[11]

A genetic link between cellular angiofibroma, mammary-type myofibroblastoma and spindle cell lipoma was demonstrated in some cases, all showing a typical loss of genetic material from the 13q14 region, as indicated by monoallelic deletion of *RB1* and *FOXO1*.[14,16] Herein we proved by FISH the heterozygote loss of *RB1* in all examined cases (n=7), thus confirming this relationship.

Hitherto, there are few reported cases of cellular angiofibroma with atypia or sarcomatous features. One case was located in the subcutis of the iliacal region and the others were localized in the vulva. Two cases contained areas of a pleomorphic liposarcoma and three of an atypical lipomatous tumor.[3,8] Our transformed case was akin to a malignant solitary fibrous tumor with pleomorphic spindle cells as characterized by Kandil et al[6] and Chen and Fletcher[8] and occurred also in the vulva. McCluggage et al[18] depicted in their series one case of the vulva with marked nuclear pleomorphism resembling symplastic change in uterine leiomyoma.

The follow-up (6 month) was as in all other cases of our series unremarkable. This finding is in agreement with the reported cases.[6,8] Therefore, the biologic significance of atypia and sarcomatous transformation remains uncertain.[8]

One of the differential diagnoses of cellular angiofibroma is solitary fibrous tumor which can also include fat and shows almost always positivity for CD34.[20] Hemangiopericytoma-like vessels were observed in a subset of cellular angiofibroma [2,3,18] and we also could find this vascular pattern in three cases. We considered this alternative possibility in one of our cases occurring in the upper eyelid and showing strong positivity for CD34. The cells were arranged in a myxoid matrix, as also described for solitary fibrous tumor.[21] However, our case consisted of evenly distributed spindle cell lipoma like cells. A prominent vasculature, typically seen in cellular angiofibroma, with partly hyalinized vessels was existent, but hemangiopericytoma-like vessels were not present in this case.

Whereas losses on chromosome 13q have been detected in solitary fibrous tumor, cytogenetic analyses to date have not found any consistent genetic abnormalities.[22-24]

Superficial myofibroblastoma of the lower female genital tract probably belongs to the morphologic spectrum of vulvovaginal stromal polyps. Variegated patterns are described including myxoid, lacelike, sieve-like, fascicular or storiform. The collagenous stroma sometimes contains thick collagen bundles.[25-27] Desmin is a discriminating marker as cellular angiofibromas are negative for this antibody.[1-3,18,25-27]

Angiomyofibroblastoma could also potentially be confused with cellular angiofibroma, because of its localization in the genital region.[28-31] It is composed of more epithelioid and often plasmocytoid cells arranged in cords and nests preferentially around vessels. Mast cells can be abundant. The tumor cells express desmin and rarely CD34 and smooth muscle actin.[28-32] Morphologically malignant cases resembling leiomyosarcoma or undifferentiated sarcoma have been reported. As described in cellular angiofibromas, none of these has metastasized to date, and one recurred 2 years later.[33,34]

Deep ("aggressive") angiomyxoma involves the pelvic region, tends to be quite large and displays an infiltrative growth with entrapment of mucosal glands, fat, muscle and nerves. The prominent vascular component shows hyalinization or hypertrophy. Myoid bundles, mostly adjacent to medium sized vessels, are a typical finding. There is a variable positivity for desmin, smooth muscle actin and CD34.[28,31,35-38]

Superficial angiomyxoma occurs among others in the genital region and possesses prominent myxoid stroma with delicate thin-walled vessels. Inflammatory cells, particularly neutrophils, are a diagnostic clue. Entrapment of an epithelial component is present in a third of the cases. This lesion may be positive for CD34.[28,39,40]

Juxtaarticular myxoma, most frequently located on the knee, arises particularly in men. This lesion shows a striking accumulation of mucinous material with poor cellularity.[41] Our cases located on the knee showed mucoid degeneration but they

were more cellular with typical features of cellular angiofibroma. In one of them we could demonstrate loss of *RB1* (case 9).

Benign nerve sheath tumors, as schwannoma and neurofibroma, contain more wavy and buckled nuclei. Discriminating S100 positivity is never described in cellular angiofibroma.[2,3] We confirmed this finding in our study group.

Expression of EMA, as seen in two out of six of our cases, could lead to the differential diagnosis of soft tissue perineurioma. The storiform, fascicular and/or whorled growth pattern of spindle cells with delicate processes are characteristic for this lesion.[42]

In summary, we could extend the clinicopathological features of cellular angiofibroma including a morphologically sarcomatous transformed variant and further extragenital localisations. Moreover, the confirmation of the genetic relationship between cellular angiofibroma, mammary-type myofibroblastoma and spindle cell lipoma suggests that these tumors are a spectrum of one entity. *RB1* FISH analysis could be an ancillary tool for supporting these diagnoses, but the specificity is uncertain by now.

References

1. Nucci MR, Granter SR, Fletcher CDM. Cellular angiofibroma: A benign neoplasm distinct from angiomyofibroblastoma and spindle cell lipoma. Am J Surg Pathol 1997;21:636-644.
2. Laskin WB, Fetsch JF, Mostofi FK. Angiomyofibroblastoma like tumor of the male genital tract: analysis of 11 cases with comparison to female angiomyofibroblastoma and spindle cell lipoma. Am J Surg Pathol 1998;22:6-16.
3. Iwasa Y, Fletcher CDM: Cellular angiofibroma: clinicopathologic and immunhistochemical analysis of 51 cases. Am J Surg Pathol 2004;28:1426-1435.
4. Garijo MF, Val-Bernal JF. Extravulvar subcutaneous cellular angiofibroma. J Cutan Pathol 1998;25:327-332.
5. Val-Bernal JF, Rubio S, Garijo MF, González-Vela MC. Extragenital subcutaneous cellular angiofibroma. Case report. APMIS 2007;115: 254-258.
6. Kandil DH, Kida M, Laub DR, Cooper K. Sarcomatous transformation in a cellular angiofibroma: a case report. J Clin Pathol 2009;62:945-947.
7. Eversole LR. Cellular angiofibroma of oral mucosa: report of two cases. Head and Neck Pathol 2009;3:136-139.
8. Chen E, Fletcher CDM. Cellular Angiofibroma with atypia and sarcomatous transformation: a clinicopathologic analysis of 13 cases. Am J Surg Pathol 2010;34:707-714.
9. Chan KW, Ghadially FN, Alagaratnam TT. Benign spindle cell tumour of the breast: a variant of spindle cell lipoma or fibroma of breast. Pathology 1984;16:331-336.
10. Wargotz ES, Weiss SW, Norris HJ. Myofibroblastoma of the breast: sixteen cases of a distinctive benign mesenchymal tumor. Am J Surg Pathol 1987;11:493-502.
11. McMenamin ME, Fletcher CDM. Mammary-type myofibroblastoma of soft tissue. A tumor closely related to spindle cell lipoma. Am J Surg Pathol 2001;25:1022-1029.
12. Dal Cin P, Sciot R, Polito P, Stas M. Lesions of 13q may occur indepently of deletion of 16q in spindle cell/pleomorphic lipomas. Histopathology 1997;31: 222-225.
13. Pauwels P, Sciot R, Croiset F, et al. Myofibroblastoma of the breast: genetic link with spindle cell lipoma. J Pathol 2000;191:282-285.
14. Maggiani F, Debiec-Rychter M, Vanbockrijck M, Sciot R. Cellular Angiofibroma: another mesenchymal tumor with 13q14 involvement, suggesting a link with spindle cell lipoma and (extra)-mammary myofibroblastoma. Histopathology 2007;51:410-412.
15. Maggiani F, Debiec-Rychter M, Verbeeck G, Sciot R. Extramammary myofibroblastoma is genetically related to spindle cell lipoma. Virchows Arch 2006;449:244-247.
16. Hameed M, Clarke K, Amer HZ, et al. Cellular angiofibroma is genetically similar to spindle cell lipoma: a case report. Cancer Genet Cytogenet 2007;177:131-134.
17. Fletcher CDM, Martin-Bates E. Spindle cell lipoma: a clinicopathological study with some original observations. Histopathology 1987;11:803-817.
18. McCluggage WG, Ganesan R, Hirschowitz L, Rollason TP. Cellular angiofibroma and related fibromatous lesions of the vulva: report of a series of cases with a morphological spectrum wider than previously described. Histopathology 2004;45:360-368.
19. Dargent JL, de Saint Aubain N, Gomez Galdon M, et al. Cellular angiofibroma of the vulva: a clinicopathological study of two cases with documentation of some unusual features and review of the literature. J Cutan Pathol. 2003;30:405-411.
20. Guillou L, Fletcher JA, Fletcher CDM, Mandahl N. Extrapleural solitary fibrous tumour and haemangiopericytoma. In: Fletcher CDM, Unni KK, Mertens F (eds). Pathology and genetics of tumors of soft tissue and bone. World Health Organization classification of tumors: IARC Press: Lyon; 2002. pp 86-90
21. De Saint Aubain Somerhausen N, Rubin BP, Fletcher CDM. Myxoid solitary fibrous tumor: a study of seven cases with emphasis on differential diagnosis. Mod Pathol 1999;12:463-471.
22. Miettinen MM, el-Rifai W, Sarlomo-Rikala M, et al. Tumor size-related DNA copy number changes occur in solitary fibrous tumors but not in hemangiopericytomas. Mod Pathol 1997;10:1194-1200.
23. Krismann M, Adams H, Jaworska M, et al. Benign solitary fibrous tumour of the thigh: morphological, chromosomal and differential diagnostics aspects. Langenbecks Arch Surg. 2000;385:521-525.

24. Park MS, Araujo DM. New insights into the hemangiopericytoma/solitary fibrous tumor spectrum of tumors. Curr Opin Oncol 2009;21:327-331.
25. Laskin WB, Fetsch JF, Tavassoli FA. Superficial cervicovaginal myofibroblastoma: fourteen cases of a distinctive mesenchymal tumor arising from the specialized subepithelial stroma of the lower female genital tract. Hum Pathol 2001;32:715-725.
26. Ganesan R, McCluggage WG, Hirschowitz L, Rollason TP. Superficial myofibroblastoma of the lower female genital tract: report of a series including tumours with a vulval location. Histopathology 2005;46:137-143.
27. McCluggage WG. Recent developments in vulvovaginal pathology. Histopathology 2009;54:156-173.
28. Nucci MR, Fletcher CDM. Vulvovaginal soft tissue tumors. Histopathology 2000;36:97-108.
29. Ockner DM, Sayadi H, Swanson PE, et al. Genital angiomyofibroblastoma. Comparison with aggressive angiomyxoma and other myxoid neoplasms of skin and soft tissue. Am J Clin Pathol 1997;107:36-44.
30. Ito M, Yamaoka H, Sano K, Hotchi M. Angiomyofibroblastoma of the male inguinal region. Arch Pathol Lab Med 2000;124:1679-1681.
31. Fletcher CDM, Tsang WY, Fisher C, et al. Angiomyofibroblastoma of the vulva: a benign neoplasm distinct from aggressive angiomyxoma. Am J Surg Pathol 1992;16:373-382.
32. Laskin WB, Fetsch JF, Tavassoli FA. Angiomyofibroblastoma of the female genital tract: analysis of 17 cases including a lipomatous variant. Hum Pathol 1997;28:1046-1055.
33. Nielsen GP, Young RH, Dickersin GR, Rosenberg AE. Angiomyofibroblastoma of the vulva with sarcomatous transformation („angiomyofibrosarcoma"). Am J Surg Pathol 1997;21:1104-1108.
34. Folpe AL, Tworek JA, Weiss SW. Sarcomatous transformation in angiomyofibroblastomas: a clinicopathological and immunohistochemical study of eleven cases. [abstract]. Mod Pathol 2001;14:12A.
35. Tsang WJ, Chan JK, Fisher C, Fletcher CD. Aggressive angiomyxoma. A report of four cases occuring in men. Am J Surg Pathol 1992;16:1059-1065.
36. Iezzoni JC, Fechner RE, Wong LS, Rosai J. Aggressive angiomyxoma in males. A report of four cases. Am J Clin Pathol 1995;104:391-396.
37. Granter SR, Nucci MR, Fletcher CDM. Aggressive angiomyxoma: reappraisal of its relationship to angiomyofibroblastoma in a series of 16 cases. Histopathology 1997;30:3-10.
38. Fetsch JF, Laskin WB, Lefkowitz M, et al. Aggressive angiomyxoma. A clinicopathologic study of 29 female patients. Cancer 1996;78:79-90.
39. Fetsch JF, Laskin WB, Tavassoli FA. Superficial angiomyxoma (cutaneus myxoma). A clinicopathologic study of 17 cases arising in the genital region. Int J Gyn Pathol 1997;16:325-334.
40. Calonje E, Guerin D, McCormick D, Fletcher CDM. Superficial angiomyxoma: clinicopathologic analysis of a series of distinctive but poorly recognized cutaneous tumors with tendency for recurrence. Am J Surg Pathol 1999;23:910-917.
41. Meis JM, Enzinger FM. Juxta-articular myxoma: a clinical and pathological study of 65 cases. Hum Pathol 1992;23:639-646.
42. Hornick JL, Fletcher CDM. Soft tissue perineurioma. Clinicopathologic analysis of 81 cases including those with atypical features. Am J Surg Pathol 2005;29:845-858.

Chapter 8

Desmoid-type fibromatosis of the head and neck region in the pediatric population. A clinicopathological and genetic study of 7 cases

Histopathology 2014 May;64(6):769-76.

Abstract

Desmoid-type fibromatosis (desmoids) is a locally aggressive (myo)fibroblastic lesion. It represents one of the more common fibrous tumors in children and adolescents. The head and neck region is more often involved when compared to adults.

We investigated the clinicopathological and genetic characteristics of seven pediatric desmoids at this anatomic site, including two cases of desmoplastic fibroma, located in the mandible. There were two females and five males with an age range from 1.5 – 8 years. Sites of the soft tissue lesions were sinonasal (n=4), and paramandibular (n=1). All cases showed typical morphology and nuclear β-catenin expression. *CTNNB1* gene sequencing, successfully performed in five cases, revealed mutations in three cases with one p.T41A (bone lesion), one p.S37A and one novel mutation, p.D32V (sinonasal soft tissue lesion each). Seven patients were treated by excision with positive margins in five cases. Follow-up, available for six patients (median, 4 years) showed no evidence of disease in four cases, slow progression in one case and recurrence with stable disease in the last case.

Our study provides evidence of genetic similarities in desmoid and desmoplastic fibroma. Additionally, we expanded the spectrum of mutations in *CTNNB1* with one for desmoid novel mutation.

Introduction

Desmoid-type fibromatosis (desmoid) is a locally aggressive (myo)fibroblastic lesion with sporadic or rarely, familial occurrence. Although more frequent in (young) adults, it represents one of the more common fibrous tumors in children and adolescents.[1] In this age group, the head and neck region is more often involved when compared to adults.[1-5]

Desmoplastic fibroma, supposed to be the morphological bone counterpart, arise also in the head and neck region of young patients, mainly within the mandible.[6-8]

Activating *CTNNB1* gene mutations (location 3p21) are found in about 85% of all sporadic desmoids, with p.T41A (threonine to alanine), p.S45F (serine to phenylalanine) and p.S45P (serine to proline) being the most frequent ones. This is similar for children and adults.[9-13] In contrast, analyses in desmoplastic fibroma have failed to show these genetic abnormalities.[6,9]

In FAP patients (Gardner Syndrome), desmoid develops on the base of inactivating *APC* gene mutations, which however also may occur in sporadic cases.[1,3,14-16]

β-catenin and APC are part of the Wnt signaling pathway, and mutations in either gene cause stabilization of β-catenin with nuclear translocation and binding to the T-cell factor/lymphoid enhancer factors (TCF/LEF) family of transcription factors. Subsequently, target genes involved in tumor biology of desmoid become activated.[9,12-15] Moreover, it was demonstrated that desmoid is derived from mesenchymal stem cells and β-catenin supports tumorigenesis by maintaining mesenchymal progenitor cells.[17]

Nuclear accumulation of β-catenin is detectable by immunohistochemistry, a useful diagnostic adjunct, but limitations have been acknowledged.[9,10,12,15,18-20]

In this study, we report the clinicopathological and genetic data of seven pediatric patients with lesions in the head and neck region including 2 intraosseous neoplasms of the mandible.

Material and Methods

The cases were retrieved from the authors' (referral) files. Clinical details and follow-up were obtained from the referring physicians. In all cases the tissue was fixed in 4% buffered formalin, routinely processed including decalcification, if needed, and embedded in paraffin; 2-4 µm thick sections were stained with hematoxylin and eosin and immunohistochemically by the labelled Streptavidin Biotin technique using a commercially available antibody against β-catenin (BD Biosciences, clone 14, dilution 1:100). Appropriate positive and negative controls were used throughout.

DNA was isolated from formalin-fixed, paraffin-embedded material (without decalcification) by proteinase K digestion and the crude DNA extract was used in a

standard PCR. The hotspot region for *CTNNB1* was amplified using primers: 5'-ATGGCCATGGAACCAGACAGA-3' and 5'-GCTACTTGTTCTTGAGTGAAGGACTG-3'. The region most frequently mutated in *APC* (NM_000038.5: amino acids 1200-1580) was amplified using the following primer pairs: 1) 5'-CAGATATTCCTTCATCACAGAAAC-3' and 5'-GGAGTATCTTCTACACAATAAGTCTG-3', 2) 5'-GCCACTTGCAAAGTTTCTTC-3' and 5'-TCACAGGATCTTCAGCTGACCT-3', 3) 5'-TCAGACGACACAGGAAGCAGAT-3' and 5'-TTTTGGGTGTCTGAGCACCACT-3', 4) 5'-AGCCAGGCACAAAGCTGTTGAA-3' and 5'-TGTCCAGGGCTATCTGGAAGATCA-3', 5) 5'-ACCATGCAGTGGAATGGTAAGTGG-3' and 5'-TGGAAGAACCTGGACCCTCTGAA-3', 6) 5'-TGGACCTAAGCAAGCTGCAGTA-3' and 5'-CTGCTCTGATTCTGTTTCATTCCCATTGT-3', 7) 5'-TCTGAGCCTCGATGAGCCATTT-3' and 5'-ACGTGATGACTTTGTTGGCATGG-3'. All PCR products were analyzed by fluorescent di-deoxysequencing.

Results

Clinical data are presented in Table 1.

Patients were two females and five males with an age range from 1.5 – 8 years (mean, 3 years; median, 2.5 years). The lesions were situated in the sinonasal (n=4) (Figure 1) and paramandibular (n=1) soft tissue. Secondary bone involvement was shown in all cases. Two tumors were located intraosseously in the mandible (Cases 6 and 7). In Case 7, extension into adjacent soft tissue was evident. Secondary bone involvement by a primarly soft tissue lesion was excluded on clinical and radiological grounds.

Table 1 Clinical Data

Case	Age/ sex	Site	Size (cm)	Treatment	Follow-up
1	1.5 y/m	sinonasal	3.1	excision, R1	3 y; rec/stable
2	1.5 y/m	sinonasal	4.5	excision, R1	3 y; NED
3	1.5 y/m	sinonasal	4	excision, R1	7 y; NED
4	2.5 y/f	sinonasal	5.3	excision, R1	5 y; NED
5	2.5y/m	paramandibular	7	excision, R0	NA; recurrence
6	8 y/f	mandible (osseous)	6	excision, R1	3 y; slow progression
7	3 y/m	mandible (osseous, soft tissue involvement)	3	resection, R0	13 y; NED

Y, years; f, female; m, male; NED, no evidence of disease; NA, information not available

Figure 1 MRI depicts a relatively circumscribed sinonasal mass (Case 1)

Six patients were treated by excision. Microscopically positive margins were documented in five cases. Case 7 was completely resected with negative margins. Follow-up, available for six patients (median, 4 years) showed no evidence of disease in four cases, slow progression in one case and recurrence with stable disease in the final case.

Case 3 was reported earlier as an odontogenic myxoma, afterwards considered to be a wrong diagnosis.[21]

Grossly, the tumors ranged in size from 3 to 7 cm and had infiltrative edges. Secondary bone involvement in the soft tissue lesions and extension into soft tissue in one bone tumor was obvious. Five tumors appeared mainly myxoid. In areas, they were firm and white with a coarse trabeculated cut surface. Two cases had only the latter appearance.

Histologically, all lesions infiltrated adjacent structures and were composed of ill-defined long fascicles of uniform (myo) fibroblasts (Figure 2) with slender tapering or stellate-shaped nuclei with a vesicular chromatin. Mitoses were found in two cases (max. 2/10 HPF). Five lesions had an extensive myxoid background and the cells were more loosely arranged (Figure 3). In areas, there were coarse keloid-like collagen

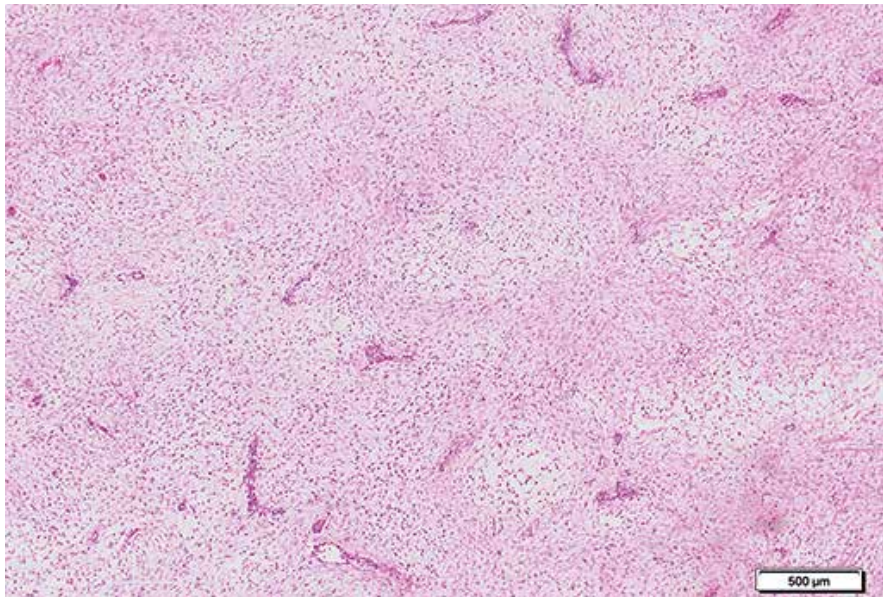

Figure 2 Low magnification shows a fascicular arrangement of uniform (myo) fibroblasts. Note the typical vascular pattern and the myxoid background

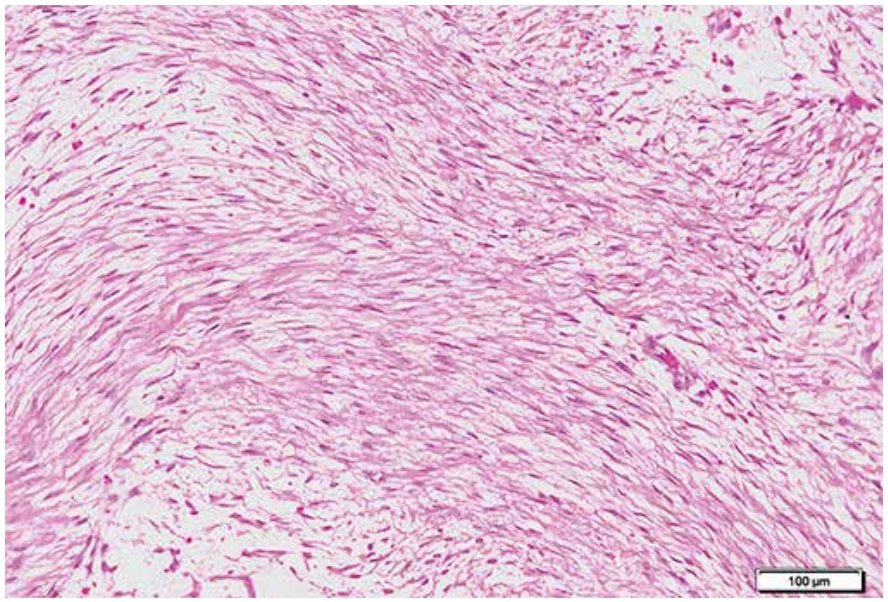

Figure 3 Loosely arranged spindle cells due to a myxoid background were seen in almost all lesions

bundles (Figure 4). The background was mainly collageneous in one soft tissue and one bone lesion (Cases 5 and 7) (Figure 5). Prominent vascularisation with parallel alignment to the fascicles was a feature in all cases (Figure 2). Some vessels were thick walled and a perivascular edema was present (Figure 6). Hemorrhage was obvious in three of the myxoid lesions (Figure 7).

Immunohistochemically, nuclear β-catenin was observed in all samples in at least 10% of the tumor cells (Table 2, Figure 8).

CTNNB1 gene sequencing showed mutations in three of five cases with one p.T41A (Case 6, mandibular lesion) (Figure 9), one p.S37A, and one p.D32V (Case 4, Figure 10) with the latter representing a hitherto undescribed mutation in desmoids. *APC* mutations were not found in the mutational cluster region in two cases with *CTNNB1* wild type. No data was obtained from the remaining two samples due to poor DNA quality (Table 2).

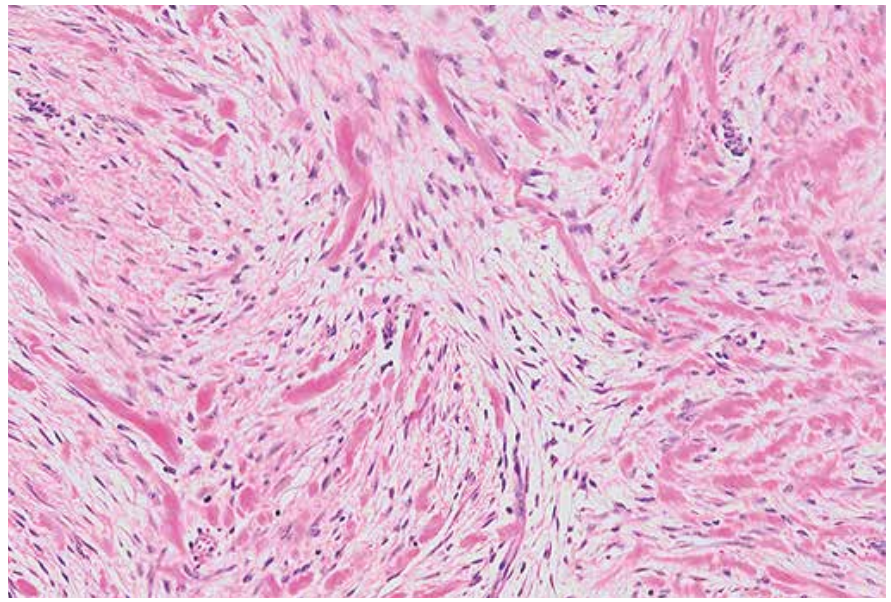

Figure 4 Thick collagen bundles were common

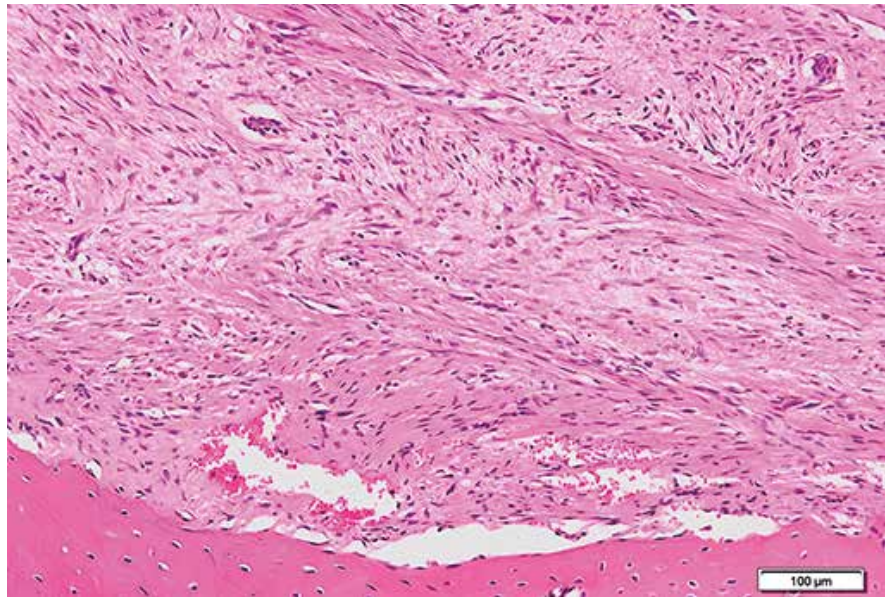

Figure 5 Desmoplastic fibroma with classical morphology. Preexistent bone is seen below

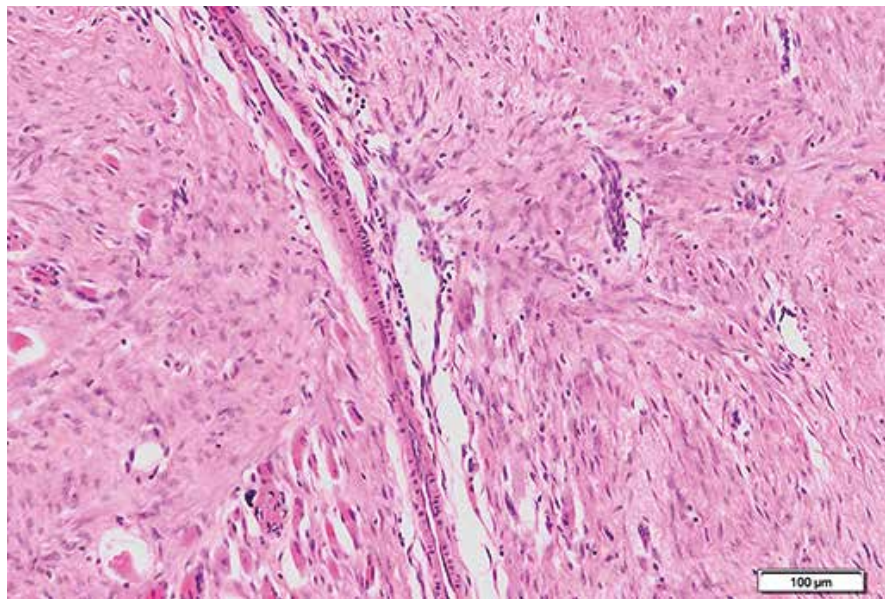

Figure 6 Typical features of a desmoid with thick walled vessels and a perivascular edema

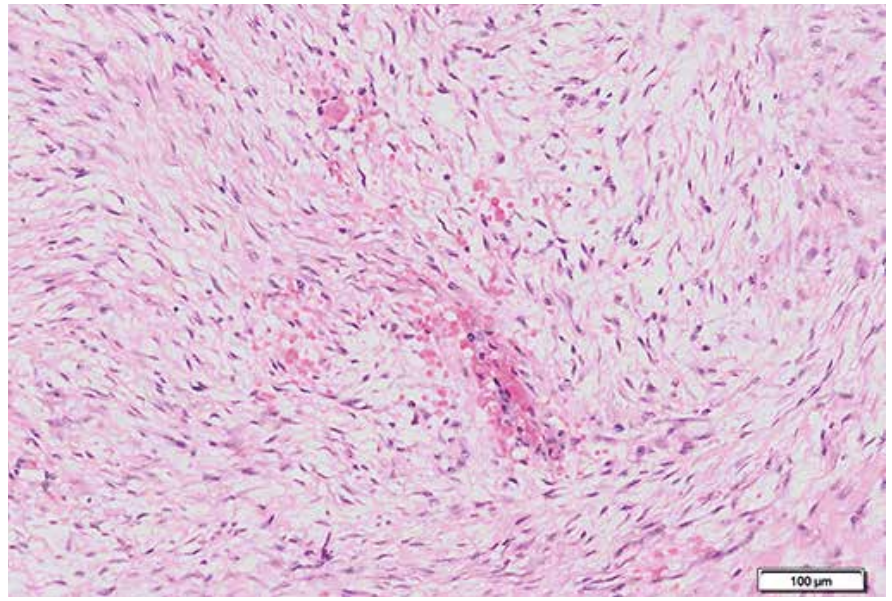

Figure 7 Erytrocyte extravasation was observed in some of the cases with myxoid background

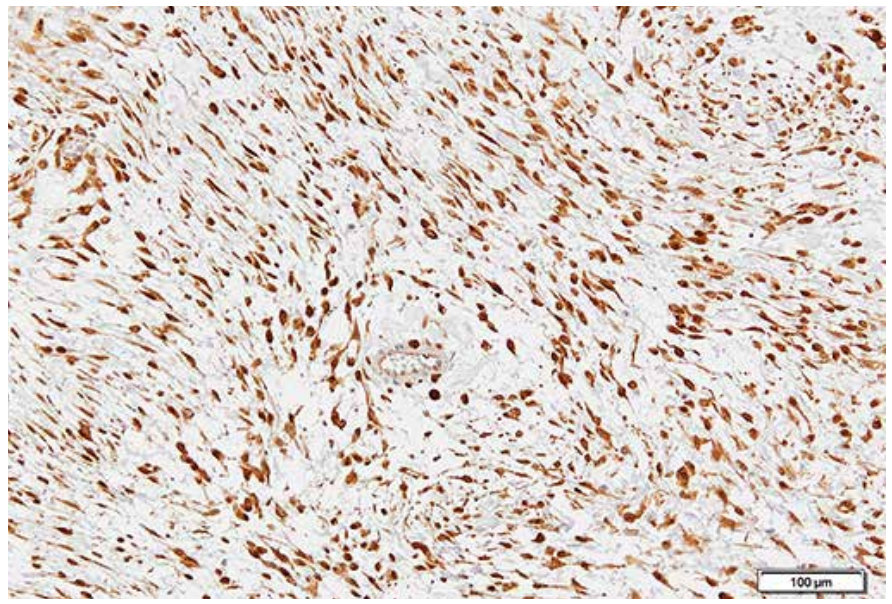

Figure 8 β-catenin was accumulated in the nuclei of all samples

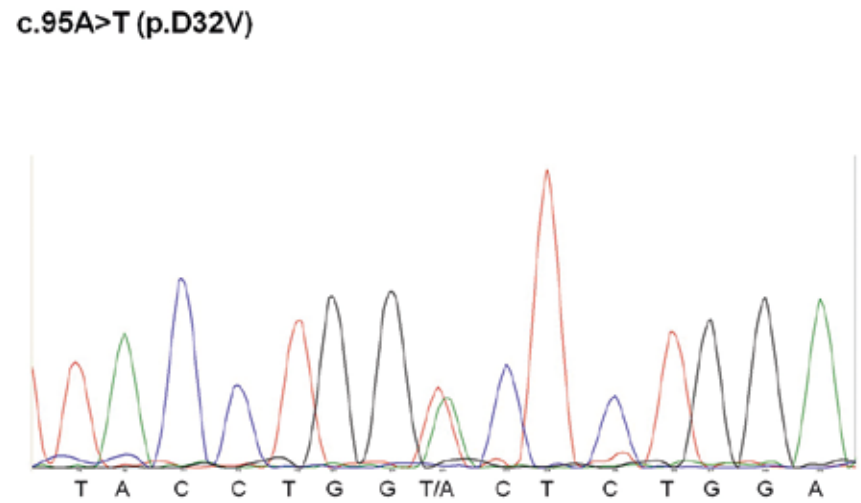

Figure 9 A classical hot spot mutation of desmoids was found in a desmoplastic fibroma of the mandible (Case 6)

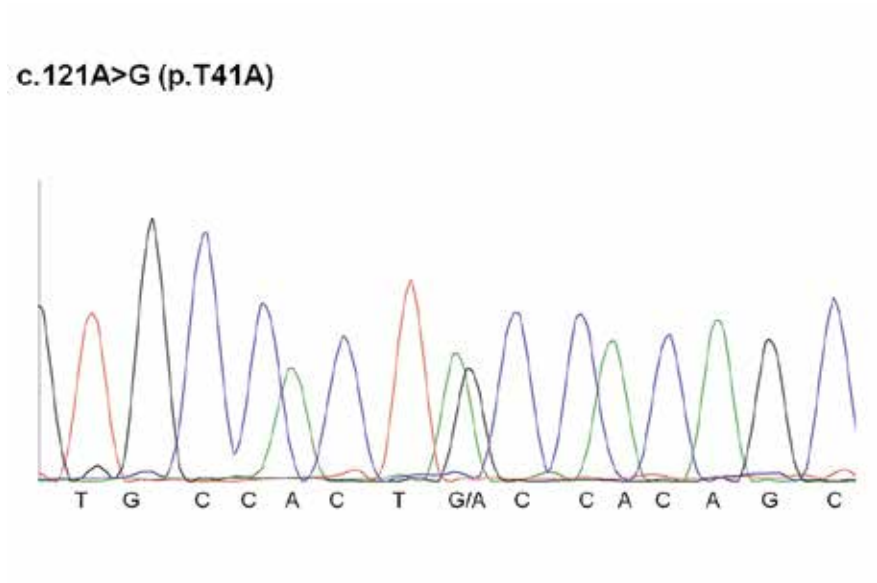

Figure 10 Novel mutation encountered in desmoid Case 4

Table 2 Immunohistochemisty and mutational analysis

Case	β-catenin (IHC)	*CTNNB1* (exon 3)
1	nuclear +/cp +	c.109T>G (p.S37A)
2	nuclear +/cp +	wild type
3	nuclear +/cp +	wild type
4	nuclear +/cp +	c.95A>T (p.D32V)
5	nuclear +/cp +	not available
6	nuclear +/cp +	c.121A>G (p.T41A)
7	nuclear +/cp +	not available

Cp, cytoplasmic

Discussion

Herein we present seven cases of desmoid of the head and neck in pediatric patients and show genetic similarities of soft tissue and bone lesions (desmoplastic fibromas). Case 6, located in the mandible bone (without soft tissue extension), harbored a p.T41A mutation, which is a classical hot spot mutation in soft tissue tumors. To our knowledge, this is the first case providing genetic evidence of the morphologically suggested relationship of desmoid and desmoplastic fibroma.[6,9] Our results disagree with those of Hauben et al.[6] Possibly, this might be due to decalcification hampering mutational analysis because of a higher degree of DNA fragmentation. As we were able to use undecalcified tissue, a better DNA quality may explain the discrepancy.

Genetic similarities between desmoids and desmoplastic fibromas were earlier demonstrated with occurrence of trisomies 8 and 20. However, these aberrations were also reported in a variety of other fibrous and fibro-osseous lesions and therefore are not specific.[22] Recently, Trombetta et al. (2012) described an exchange between chromosome arms 11q and 19p in a case of desmoplastic fibroma. Whether this is a specific and consistent abnormality remains yet unknown.[23]

In two sinonasal soft tissue lesions, we found two point mutations in codon 32 and 37 (exon 3) of *CTNNB1* – p.D32V (aspartic acid to valine) and p.S37A (serine to alanine). P.D32V has not yet been reported in desmoids while p.S37A was described in a single case in a recently published large series.[13] Other rare mutations, p.I35S and p.T41I, have been detected earlier in cases of head and neck desmoids possibly suggesting that heterogeneous mutations are associated with this site.[24,25]

In contrast, a more homogeneous mutation pattern has been demonstrated in other localizations with p.S45F overrepresentation in tumors of the extremities and a

prevalence of p.T41A in mesenteric lesions.[10,12,13,20,26] Additional novel point mutations in codon 32, 33, 34, and 45 were detected in only about 3% of desmoids.[13,20] In this context, Huss et al stressed the impact of chosen methods to find unexpected mutations.[20]

None of our patients showed a mutation in the *APC* gene nor were they known to bear familial adenomatous polyposis (FAP)-syndrome. This is not surprising when we consider that FAP related desmoids have a prevalence of around 70% for the abdominal site.[27,28] Also in sporadic desmoids with *APC* mutations, the abdomen is a predilection site.[29,30] A genotype-phenotype correlation has been reported in FAP patients as well with mainly different mutations in extremities and the abdominal site.[28]

(Myo) fibroblastic lesions entering the differential diagnosis of desmoids in the pediatric population at this anatomic site are cranial/nodular fasciitis, low-grade fibromyxosarcoma, low-grade myofibroblastic sarcoma, lipofibromatosis, malignant peripheral nerve sheath tumor, myofibroma, leiomyosarcoma, inflammatory myofibroblastic tumor, and hypertrophic scar.

Cranial/nodular fasciitis is an important alternative diagnostic possibility, especially in cases with loosely arranged myxoid areas. Shorter fascicles, intermingled inflammatory cells and erythrocyte extravasation are characteristic for this lesion.[1] Erythrocyte extravasation can also be present in desmoids as we found in three of our cases. But the more thick-walled vessels and degenerated skeletal muscle at the edge of desmoids are discriminating.[1] Moreover, nuclear expression of β-catenin has not been attributed to nodular fasciitis.[19] When taking genetics into account, *USP6* rearrangement was found in all investigated nodular/cranial fasciitis cases suggesting that *USP6*-FISH is a useful diagnostic adjunct in difficult cases.[32] In contrast, cranial fasciitis cases with mutations in *CTNNB1* and *APC,* probably represented examples of desmoid, as illustrated and discussed by the authors.[25]

Another differential diagnosis is that of low-grade fibromyxoid sarcoma (LGFMS).[33] LGFMS also affects children and may occur in the head and neck region[34,35] and a more fascicular pattern of the bland looking fibroblasts can resemble desmoid.[34] As recently demonstrated, MUC4 is a specific immunohistochemical marker for this tumor and *FUS-CREB3L2/FUS-CREB3L1* fusions are the genetic findings in the majority of cases.[35-37] Regarding nuclear expression of β-catenin, there are discrepant results.[9,18]

Low-grade myofibroblastic sarcoma (LGMS) characterized by fibromatosis-like features has a predilection for the head and neck region but children are rarely affected. Histologically, the fascicles are more cellular and the myofibroblasts possess atypical nuclei with hyperchromasia.[8] Presence of nuclear expression of β-catenin does not rule out this tumor-type[19] but described genetic aberrations are quite different from desmoid.[8,38]

Lipofibromatosis, a rare tumor in childhood, is reported in the head and neck area and shows a fatty component between fibrous streaks. The latter closely resembles desmoids, but there is no nuclear reaction for β-catenin and the genetic background

seems to be different with a complex translocation described in a single case.[8]

Malignant peripheral nerve sheath tumor (MPNST) is outside the NF1(neurofibromatosis type 1) -setting less likely. Nuclear atypia can be mild in MPNST and desmoids are known for traces of S-100 protein positivity which can be confused with morphologically low grade MPNST.[39] Nuclear β-catenin labeling has been reported.[40]

Myofibroma, a relatively common tumor in childhood, has a predilection for the head and neck, and bone involvement has been reported. The biphasic appearance of myoid nodules with a distinctive myxohyaline matrix and hemangiopericytoma-like intervening areas denotes this lesion.[1] Moreover, there is no nuclear β-catenin expression.[18]

Leiomyosarcoma rarely occurs in children although the head and neck region can be involved.[41] Low-grade lesions may be confused with desmoids probably based on the rare expression of desmin in addition to SMA.[8] H-caldesmon demonstrates smooth muscle differentiation and is not expressed in myofibroblastic lesions[42] and nuclear β-catenin accumulation has not been observed in leiomyosarcomas.[18]

Extrapulmonary inflammatory myofibroblastic tumor (IMT) is a plausible consideration when fascicles and a prominent collagenous background are present[43] but the prominent inflammatory reaction throughout the lesion is a distinguishing mark.[39] ALK expression and rearrangements (2p23) are reported in 50% - 70% of the cases.[44-46] Furthermore, ALK staining is negative in desmoids[47] and, there is no nuclear β-catenin expression in IMT.[19]

Infantile fibrosarcoma almost exclusively occurs in the first year of life and the head and neck is a possible site. This lesion, composed of fascicles or sheets of more primitive myofibroblasts with enlarged nuclei, often shows a high mitotic activity. The immunophenotype is variable and both SMA and desmin can be positive. *ETV6-NTRK3* fusion is the molecular key to the diagnosis.[8,39,48]

Also a hypertrophic scar can mimic desmoid, and sometimes, this is an important differential diagnosis, especially in surgically treated desmoid patients. However, a hypertrophic scar lacks the fascicular and vascular pattern of desmoids, and nuclear β-catenin staining is also absent.[1,10]

Regarding treatment, one of the main problems in managing desmoids is their high propensity to recur after initial treatment, but this is unpredictable and stable disease and even regression are also possible outcomes.

Therefore, surgery as the mainstay is under debate and a wait-and-see policy as initial strategy in a none-life threatening site and in the absence of marked progression is being adopted. Furthermore, function-sparing intervention should be preferred to aggressive surgery aiming at negative margins.[2,4,16,49]

Whether age has a prognostic impact is controversial.[4,16,50] Two studies of the pediatric population showed that younger age (<10 years) is a favorable prognostic factor.[4,50]

It also seems that lesions in the head and neck have a relative good prognosis particularly when treated by surgery[2,4,50] and microscopic extension into the margins does not impair the result.[2,4,16,26] Our study supports these findings. However, when vital structures are involved, the risk of morbidity and mortality are high in this region.[2,49,51] An alternative therapeutic perspective could be small molecule inhibitors of the Wnt/ β-catenin signaling.[52]

In summary, the head and neck region is a preferential site of desmoids in the pediatric population often associated with a good prognosis. Comparable lesions in the bone (desmoplastic fibromas) are genetically related as we could demonstrate for the first time. Regarding the broad spectrum of differential diagnosis, morphology is still the diagnostic cornerstone. Mutational analysis of *CTNNB1* can be a confirming adjunct in most of the cases since morphologic mimics are negative.[13,53] Finally, we expanded the spectrum of mutations in this gene by describing one novel and one rare mutation outside the regular hot spots as currently listed for this tumor.

References

1. Coffin CM, Alaggio R. Fibroblastic and myofibroblastic tumours in children and adolescents. Pediatr Dev Pathol 2012;15(Suppl):127-180.
2. Sharma A, Ngan BY, Sándor GK, et al. Pediatric aggressive fibromatosis of the head and neck: a 20-year retrospective review. J Pediatr Surg 2008;43:1596-1604.
3. Murphey MD, Ruble CM, Tyszko SM, et al. From the archives of the AFIP. Musculoskeletal fibromatoses: Radiologic-pathologic correlation. Radiographics 2009;29:2143-2176.
4. Oudot C, Orbach D, Minard-Colin V, et al. Desmoid fibromatosis in pediatric patients: management based on a retrospective analysis of 59 patients and a review of the literature. Sarcoma 2012;2012:475202.
5. Gnepp DR, Henley J, Weiss S, Heffner D. Desmoid fibromatosis of the sinonasal tract and nasopharynx. Cancer 1996;78:2572-2579.
6. Hauben EI, Jundt G, Cleton-Jansen AM, et al. Desmoplastic fibroma of bone: an immunohistochemical study including β-catenin expression and mutational analysis for β-catenin. Hum Pathol 2005;36:1025-1030.
7. Unni KK, Inwards CY (eds). Desmoplastic fibroma. In: Dahlin's Bone tumors. 6th edn. Philadelphia, PA: Lippincott Williams & Wilkins; 2010;175-178.
8. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F (eds). WHO classification of tumours of soft tissue and bone. Lyon, France: IARC;2013.
9. Amary MF, Pauwels P, Meulemans E, et al. Detection of β-catenin mutations in paraffin-embedded sporadic desmoid-type fibromatosis by mutation-specific restriction enzyme digestion (MSRED): an ancillary diagnostic tool. Am J Surg Pathol 2007;31:1299-1309.
10. Lazar AJ, Tuvin D, Hajibashi S, et al. Specific mutations in the beta-catenin gene (CTNNB1) correlate with local recurrence in sporadic desmoid tumors. Am J Pathol 2008;173:1518-1527.
11. Salas S, Chibon F, Noguchi T, et al. Molecular characterization by array comparative genomic hybridization and DNA sequencing of 194 desmoid tumors. Genes Chromosomes Cancer 2010;49:560-568.
12. Wang WL, Nero C, Pappo A, et al. CTNNB1 genotyping and APC Screening in pediatric desmoid tumors: a proposed algorithm. Pediatr Dev Pathol 2012;15:361-367.
13. Le Guellec S, Soubeyran I, Rochaix P, et al. *CTNNB1* mutation analysis is a useful tool for the diagnosis of desmoid tumors : a study of 260 desmoid tumors and 191 potential morphologic mimics. Mod Pathol 2012;25:1551-1558.
14. Alman BA, Li C, Pajerski ME, et al. Increased β-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumors). Am J Pathol 1997;151:329-334.
15. Tejpar S, Nollet F, Li C, et al. Predominance of beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor). Oncogene 1999;18:6615-6620.
16. Salas S, Dufresne A, Bui B, et al. Prognostic factors influencing progression-free survival determined from a series of sporadic desmoid tumors: a wait-and-see policy according to tumor presentation. J Clin Oncol 2011;29:3553-3558.
17. Wu C, Nik-Amini S, Nadesan P, et al. Aggressive fibromatosis (desmoid tumor) is derived from mesenchymal progenitor cells. Cancer Res 2010;70;7690-7698.
18. Bhattacharya B, Dilworth HP, Iacobuzio-Donahue C, et al. Nuclear β-catenin expression distinguishes deep fibromatosis from other benign and malignant fibroblastic and myofibroblastic lesions. Am J Surg Pathol 2005;29;653-659.
19. Carlson JW, Fletcher CDM. Immunohistochemistry for β-catenin in the differential diagnosis of spindle cell lesions: analysis of a series and review of the literature. Histopathology 2007;51:509-514.
20. Huss S, Nehles J, Binot E, et al. β-catenin (CTNNB1) mutations and clinicopathologic features of mesenteric desmoid-type fibromatosis. Histopathology 2013;62:294-304.
21. Fenton S, Slootweg PJ, Dunnebier EA, Mourits MP. Odontogenic myxoma in a 17-month-old child: a cases report. J Oral Maxillofac Surg 2003;61:734-736.
22. Bridge JA, Swarts SJ, Buresh C, et al. Trisomies 8 and 20 characterize a subgroup of benign fibrous lesions arising in both soft tissue and bone. Am J Pathol 1999;154: 729-733.

23. Trombetta D, Macchia G, Mandahl N, et al. Molecular genetic characterization of the 11q13 breakpoint in a desmoplastic fibroma of bone. Cancer Genet 2012;205:410-413.
24. Kattentidt Mouravieva AA, Geurts-Giele IR, de Krijger RR, et al. Identification of familial adenomatous polyposis carriers among children with desmoid tumours. Eur J Cancer 2012;48:1867-1874.
25. Rakheja D, Cuningham JC, Mitui M, et al. A subset of cranial fasciitis is associated with dysregulation of the Wnt/β-catenin pathway. Mod Pathol. 2008;21:1330-1336.
26. Colombo C, Miceli R, Lazar AJ, et al. CTNNB1 45F is a molecular prognosticator of increased postoperative primary desmoid tumor recurrence. Cancer 2013; doi: 10.1002/cncr.28271.
27. Fallen T, Wilson M, Morlan B, Lindor NM. Desmoid tumors – a characterization of patients seen at Mayo Clinic 1976-1999. Fam Cancer 2006;5:191-194.
28. Nieuwenhuis MH, Casparie M, Mathus-Vliegen LMH, et al. A nation-wide study comparing sporadic and familial adenomatous polyposis-related desmoid-type fibromatoses. Int J Cancer 2011;129:256-261.
29. Escobar C, Munker R, Thomas JO, et al. Update on desmoid tumors. Ann Oncol 2012;23:562-569.
30. Giarola M, Wells D, Mondini P, et al. Mutations of adenomatous polyposis coli (APC) gene are uncommon in sporadic desmoid tumors. Br J Cancer 1998;78:582-587.
31. Robanus-Maandag E, Bosch C, Amini-Nik S, et al. Familial adenomatous polyposis-associated desmoids display significantly more genetic changes than sporadic desmoids. PloS One 2011;6:e24354.
32. Erickson-Johnson MR, Chou MM, Everts BR, et al. Nodular fasciitis: a novel model of transient neoplasia induced by *MYH9-USP6* gene fusion. Lab Invest 2011;91:1427-1433.
33. Evans HL. Low-grade fibromyxoid sarcoma. A report of 12 cases. Am J Surg Pathol 1993;17:595-600.
34. Billings SD, Giblen G, Fanburg-Smith JC. Superficial low-grade fibromyxoid sarcoma (Evans Tumor). A clinicopathologic analysis of 19 cases with a unique observation in the pediatric population. Am J Surg Pathol 2005;29:204-210.
35. Doyle LA, Möller E, Dal Cin P, et al. MUC4 is a highly sensitive and specific marker for low-grade fibromyxoid sarcoma. Am J Surg Pathol 2011;35:733-741.
36. Panagopoulos I, Storlazzi CT, Fletcher CD, et al. The chimeric FUS/CREB3L2 gene is specific for low-grade fibromyxoid sarcoma. Genes Chromosomes Cancer 2004; 40:218-228.
37. Mertens F, Fletcher CD, Antonescu CR, et al. Clinicopathologic and molecular genetic characterization of low-grade fibromyxoid sarcoma, and cloning of a novel *FUS/CREB3L1* fusion gene. Lab Invest 2005;85:408-415.
38. Fletcher CD, Dal Cin P, de Wever I, et al. Correlation between clinicopathological features and karyotype in spindle cell sarcomas. A report of 130 cases from the CHAMP study group. Am J Pathol 1999;154:1841-1847.
39. Fletcher CDM. Soft tissue tumors. In: Fletcher CDM (ed). Diagnostic histopathology of tumors. 3rd edn. Philadelphia, PA: Churchill Livingstone Elsevier; 2007.
40. Kuhnen C, Herter P, Müller O, et al. Beta-catenin in soft tissue sarcomas: expression is related to proliferative activity in high-grade sarcomas. Mod Pathol 2000;13:1005-1013.
41. de Saint Aubain Somerhausen N, Fletcher CD. Leiomyosarcoma of soft tissue in children: clinicopathologic analysis of 20 cases. Am J Surg Pathol 1999;23:755-763.
42. Perez-Montiel MD, Plaza JA, Dominguez-Malagon H, Suster S. Differential expression of smooth muscle myosin, smooth muscle actin, h-caldesmon, and calponin in the diagnosis of myofibroblastic and smooth muscle lesions of skin and soft tissue. Am J Dermatopathol 2006;28:105-111.
43. Coffin CM, Watterson J, Priest JR, Dehner LP. Extrapulmonary inflammatory myofibroblastic tumor (inflammatory pseudotumor). Am J Surg Pathol 1995;19:859-872.
44. Griffin CA, Hawkins AL, Dvorak C, et al. Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. Cancer Res 1999;59:2776–2780.
45. Coffin CM, Patel A, Perkins S, et al. ALK1 and p80 expression and chromosomal rearrangements involving 2p23 in inflammatory myofibroblastic tumor. Mod Pathol 2001;14:569-576.
46. Coffin CM, Hornick JL, Fletcher CDM. Inflammatory myofibroblastic tumor. Comparison of clinicopathologic, histologic, and immunohistochemical features including ALK expression in atypical and aggressive cases. Am J Surg Pathol 2007;31:509-520.

47. Cook JR, Dehner LP, Collins MH, et al. Anaplastic lymphoma kinase (ALK) expression in the inflammatory myofibroblastic tumor. Am J Surg Pathol 2001;25: 1364-1371.
48. Miettinen M. Infantile fibrosarcoma (congenital fibrosarcoma). In: Miettinen M (ed). Modern soft tissue pathology. Tumors and non-neoplastic conditions. NY, NY: Cambridge University Press;2010;301-304.
49. Faulkner LB, Hajdu SI, Kher U, et al. Pediatric desmoid tumor: retrospective analysis of 63 cases. J Clin Oncol 1995;13:2813-2818.
50. Meazza C, Bisogno G, Gronchi A, et al. Aggressive fibromatosis in children and adolescents: the Italian experience. Cancer 2010;116:233-240.
51. Gnepp DR, Henley J, Weiss S, Heffner D. desmoid fibromatosis of the sinonasal tract and nasopharynx. Cancer 1996;78:2572-2579
52. Voronkov A, Krauss S. Wnt/beta-catenin signaling and small molecule inhibitors. Curr Pharm Des 2013;19:634-664.
53. Colombo C, Bolshakov S, Hajibashi S, et al. 'Difficult to diagnose'desmoid tumours: a potential role for CTNNB1 mutational analysis. Histopathology 2011;59:336-340.

Chapter 9

Summarizing discussion

Summarizing discussion

There has been huge progress in our understanding of soft tissue tumors including clinicopathologic characterization of new entities, major revisions of the classifications and, most especially, recognition of the molecular genetic basis of many of these lesions, yielding not only pathogenetic insights but also a range of valuable new diagnostic tools. This has direct practical relevance to surgical pathologists and, consequently, for patients and clinicians as well.[1]

In this respect, we discuss herein rearrangements and gene fusions in different tumor types with the main topic being *EWSR1* rearrangement in myoepithelial tumors. Additionally, changes of important tumor suppressor genes in epithelioid sarcoma and cellular angiofibroma, and mutations in the oncogene *CTNNB1,* known to be involved in the development of desmoid fibromatosis, are debated.

Rearrangements involving *EWSR1* (22q12) were first described in Ewing sarcoma. However, it has become increasingly clear that *EWSR1* is a "promiscuous" gene, with rearrangements involving a number of different partner genes identified in a variety of clinicopathological distinct soft tissue tumors including soft tissue myoepithelial tumors, extraskeletal myxoid chondrosarcoma, desmoplastic small round cell tumor, clear cell sarcoma, angiomatoid fibrous histiocytoma, primary pulmonary myxoid sarcoma, clear cell sarcoma-like gastrointestinal tumor, and a subset of myxoid liposarcomas, and low-grade fibromyxoid sarcomas/sclerosing epithelioid fibrosarcomas.[2,3] *EWSR1* rearrangements have also been identified in non-mesenchymal tumors, hyalinizing clear cell carcinoma of the salivary gland and clear cell odontogenic carcinoma.[2,4,5] The issue of EWSR1 rearrangement has been further explored in **chapters 2 to 5**.

Cutaneous myoepithelial tumors and their soft tissue counterparts represent points along a clinicopatholological spectrum that was extended to include myoepithelial tumors of the bone and some visceral sites (salivary glands and lung). They can be benign or malignant and histomorphological criteria have been proposed to define the dignity. The suggested morphological relationship of tumors at the mentioned sites was supported by evidence of *EWSR1* rearrangement in a subset of cases with the same frequency (ca. 50%) in skin, as shown in our study (**chapter 2**), and soft tissue tumors. To date, four different fusion partners were found – *POUF5F1, PBX1, ZNF444,* and *ATF1* with the lowest frequency for the latter two genes. *EWSR1-ATF1* has been detected in one of our soft tissue tumors (**chapter 3**).

Hitherto known alternative rearranged genes to EWSR1 are *PLAG1* and exceptionally *FUS*.[6,7,8]

Extraskeletal myxoid chondrosarcoma is a malignant tumor of the deep soft tissue and a morphologic mimic of myoepithelial tumors. Since *EWSR1* rearrangement

occurs in both entities, the specific rearrangement of *NR4A3* in extraskeletal myxoid chondrosarcoma is the most reliable discriminating feature (**chapter 4**). The distinction is important because of the different biological behavior. Whereas myoepithelial carcinomas (malignant myoepithelial tumors) are often aggressive tumors, especially in children, extraskeletal myxoid chondrosarcoma has a prolonged clinical course. This can have impact on possible tailored treatment modalities, especially in the children population.[9]

Another potential histomorphologic mimic of myoepithelial tumors is the myxoid variant of epithelioid sarcoma. The absence of the INI1 protein is a key immunohistochemical finding in epithelioid sarcomas since it is lost in most of the cases (ca. 90%) in comparison to benign myoepithelial tumors in which INI1 is retained. This was also a finding of our studies (**chapter 2, chapter 5**).

In epithelioid sarcoma, underlying aberrations of *INI1,* a tumor suppressor gene located on the long arm of chromosome 22, are only in part elucidated with intragenetic changes (deletions and mutations) in a subset of cases. Therefore, alternative epigenetic inactivating mechanisms of *INI1* are discussed. The loss of the INI1 protein has been established as a useful diagnostic marker for this tumor type. However, it has to be mentioned that there is overlap with other entities, e.g. myoepithelial carcinomas and epithelioid peripheral nerve sheath tumors. The genetic background of the latter two entities is still unknown.

In conclusion, our study provides evidence of a genetic relationship of myoepithelial tumors (benign and malignant) at different sites, especially in skin and soft tissue, and expands the spectrum of lesions harboring *EWSR1* rearrangements. The occurrence of *EWSR1* rearrangements in only ca. 50% of tumors with different fusion partners demonstrate the genetic heterogeneity of these tumors possessing also a protean morphological spectrum. Distinction from morphologic mimics as extraskeletal myxoid chondrosarcoma and epithelioid sarcoma is important because of the different biology and clinical behavior.

The *EWSR1-ATF1* fusion has been detected in different benign and malignant entities as clear cell sarcoma, angiomatoid fibrous histiocytoma, and myoepithelioma providing arguments that histomorphology and immunohistochemistry are necessary to make the correct diagnosis.

In **chapter 6**, we reported on the rare entity of chondroid lipomas. These deep-seated benign adipose tumors are genetically characterized by a recurrent translocation t(11;16)(q13;p13) with the corresponding fusion gene *C11orf95-MKL2.* The rarity and peculiar morphology of this tumor type with a chondromyxoid matrix can cause diagnostic confusion with being the most important differential diagnoses extraskeletal myxoid chondrosarcoma and myxoid liposarcoma. Myoepithelial tumors enter also the differential diagnosis, as keratin and S100 expression are overlapping immunohistochemical features. The fusion gene *C11orf95-MKL2* was recently found

and validated in our study. While the MKL2 protein is known to be involved in chromatin remodeling and transcription, the function of C11orf95 is as yet unknown. In conclusion, this recently detected genetic characteristic could be a useful diagnostic adjunct in morphological difficult cases to avoid overtreatment. Furthermore, it shows the upcoming myriad of fusion genes disclosed by introduction of a variety of new molecular detection methods.

The next chapter, **Chapter 7**, shows deletion of the tumorsuppressor gene *RB1* as a recurrent genetic aberration in cellular angiofibroma. This benign tumor, mostly occurring in the inguinogenital region of adults is morphologically, immunohistochemically and genetically related to spindle cell lipoma and mammary-type myofibroblastoma. This is supported by the shown loss of *RB1*. Due to their relationship one could argue that these lesions represent a spectrum of one entity with different localizations rather than different tumor types.

In **chapter 8**, we characterized desmoid-type fibromatosis occurring in the head and neck region of the pediatric population. Head and neck desmoids are disproportionately common in children and are clinically characterized by a good prognosis independent on the result of surgery. Desmoids have a recurrent mutation of *CTNNB1* resulting in nuclear accumulation of beta-catenin that activates the wnt-signaling and is, at least in part, responsible for tumorigenesis. From the diagnostic point of view, immunohistochemical detection of nuclear accumulation beta-catenin and mutational analysis of the corresponding gene can support the diagnosis, even in small biopsies. We found in our cohort one novel and one rare mutation in this gene suggesting a more heterogeneous mutation pattern in exon 3 of *CTNNB1* at this anatomic site. Furthermore, one bone lesion, called desmoplastic fibroma, located in the mandibular bone, showed a hot spot mutation. Therefore, a morphologically suggested link with desmoids was genetically confirmed in our study.

The last two chapters show that implementation of genetic characteristics of soft tissue (and bone) tumors can influence morphological classifications forming families of tumors with the same biologic background.

All here described molecular changes are consistent and specific findings and their detection can be used as ancillary diagnostic tools for pathologists in making a proper diagnosis and consequently, helping clinicians in managing treatment for patients.

New insight in pathobiology of the decribed lesions is a step towards tailored treatment possibilities especially in sarcomas. The genetic characterization of benign lesions helps to simplify classifications when relationships between tumors are obvious as we could demonstrate for cellular angiofibromas and desmoid fibromatosis.

Future perspectives

Until now, and as reflected in the current *WHO classification of Tumours of Soft Tissue and Bone* (2013),[10] microscopic morphology, supplemented where relevant by ancillary techniques (immunohistochemistry and molecular tests) has remained the cornerstone of the classification and diagnosis of soft tissue (and bone) tumors. The underlying principle is that a tumor is defined by its cell of origin and its state of differentiation. It is remarkable that these well-established technologies continue to facilitate the clinically useful definition of previously unrecognized tumor-types on a regular basis.[11]

The classification of tumors nowadays is based on the definition of so-called entities. An entitiy is seen as a separate disease and is defined on morphological, immunophenotypical, genetic and clinical features. Ideally, an entity has a specific set of criteria and the diagnosis gives clues on prognosis and treatment for the patient and the clinician.

The last couple of years, genetic characterization of soft tissue tumors has become a rapidly growing area and findings that are characteristic for individual entities can be very useful in understanding their pathobiology, including the detection of pathways related to tumorigenesis and in the identification of novel diagnostic markers (e.g. gene mutations or amplifications and fusion genes). Methods for this used in daily diagnostic practice are PCR and sequencing of the gene products (Sanger method, gene scanning and next generation sequencing) and fluorescence in situ hybridization. This will radically change the way how soft tissue tumors get diagnosed: not only a cell of origine, state of differentiation and disease entity are important for the classification of a given tumor, but also the molecular changes will be characterised. Already nowadays we see that this lead to targeted treatments that are directed to the altered molecular pathway. Consequently, diagnosis and treatment of soft tissue tumors enter the area of precision medicine.

What will be the consequenties of this large scale introduction of genetic techniques in soft tissue tumor diagnosis? It can be expected that next-generation sequencing will reveal a much higher incidence of tumor-specific aberrations than observed until now. These can directly be used for improved classification of tumors as shown in this thesis. Even more important may be the question which of these changes lead to altered gene function. To solve this, characterisation of tumor tissue at the RNA and protein level will help to distinguish biologically relevant abnormalities from alterations that do not have any biological significance. The data need to be combined with clinical data in order to provide a clinical relevant picture of the tumor. Some of the involved genes and active pathways are targets of drugs already approved for therapy of other conditions and it has to be explored whether they represent possible treatment strategies for soft tissue tumors as well.[12] It can be expected that many

more targets will be discovered and become applicable in the near future, and this will completely alter the therapeutic approach for patients with soft tissue tumors. Physicians of the future would extend their diagnostic strategy by elucidating the exact molecular basis of each patient's disease and subsequently apply a specifically targeted intervention if available. At the heart of this radically different model for patient care lies the application of high-throughput genomic technologies in individual patients.[13] However, the next couple of years, histomorphology will still be the first step to characterize a given tumor at least for representativity and to exclude unexpected (secondary or reactive) lesions. In addition, different tumor-types share identical genetic aberrations (e.g. gene fusions), making histological examination essential because of different treatment modalities for different tumor types.

Because of their knowledge in characterizing tissue, pathologists have to evaluate genetic and molecular information with a great appreciation for cellular context taking into account the rich interplay between genes, cells and the microenvironment.[13] Therefore, they need to interact with molecular geneticists and bioinformaticians to provide surgeons and oncologists the information they need for proper patient management.

It is therefore clear that genome-based research on diseased tissues should rely on both histopathological and molecular classification systems.[13]

The accrual of appropriate tissue samples is one of the greatest challenges in genomic research and the development of molecular diagnostic tools. For example, many genome-based technologies work best on frozen tissue samples, and a definite validation of a particular disease marker or prognostic indicator depends on its detection in patients participating in prospective clinical trials. Because pathologists are the gateway to high-quality, well characterized tissue specimens, they are in a unique position to help push these studies forward.[13] Therefore, all soft tissue tumors need to be evaluated in a laboratory that provide state of the art biobanking.

Molecular tools will undoubtedly improve the diagnosis and treatment of illness in years to come, but the availability of advanced new technologies is not the same as recognizing, understanding and thereby better characterizing a given disease.[13]

The goal of personalized medicine is to identify virtually all of the targetable genetic and also epigenetic abnormalities (e.g. DNA methylation and histone modification) playing a role in the initiation and progression of a patient's tumor. To develop targeted treatment for different cancer types, we also need a more sophisticated understanding of tumor-specific antigens and epigenetic changes as mentioned above. There likely will be many surprises along the way, and paradigms will be discarded. Nevertheless, the goal will always be the same – to treat disease and benefit the patient.[12] This can be supported and improved by panel discussions and national and international networks, especially for rare cancers as are sarcomas. Additionally, further research including animal model systems is mandatory.

Conclusions

In the era of tumor-specific targeted therapies it is essential to correctly identify sarcoma types and to exclude mimics from the diagnosis. The rapidly evolving molecular approaches are helpful in this process and wil get a more central role. By careful correlation of all aspects of a case – clinical, radiological, histologic, immuno-phenotypic, and molecular – an accurate diagnosis and, consequently, adequate treatment can be achieved.[13]

References

1. Long course book USCAP 2013
2. Boland JM, Folpe AL. Cutaneous neoplasms showing EWSR1 rearrangement. Adv Anat Pathol 2013; 20:75-85.
3. Doyle LA, Wang WL, Dal Cin P, et al. MUC4 is a sensitive and extremely useful marker for sclerosing epithelioid fibrosarcoma: association with FUS gene rearrangement. Am J Surg Pathol 2012;36:1444-1451.
4. Antonescu CR, Katabi N, Zhang L, et al. EWSR1-ATF1 fusion is a novel and consistent finding in hyalinizing clear-cell carcinoma of salivary gland. Genes Chromosomes Cancer 2011;50:559-570.
5. Bilodeau EA, Weinreb I, Antonescu CR, et al. Clear cell odontogenic carcinomas show EWSR1 rearrangements: a novel finding and a biological link to salivary clear cell carcinomas. Am J Surg Pathol 2013;37:1001-1005.
6. Antonescu CR, Zhang L, Shao SY, et al. Frequent PLAG1 gene rearrangements in skin and soft tissue myoepithelioma with ductal differentiation. Genes Chromosomes Cancer 2013;52:675-682.
7. Bahrami A, Dalton JD, Krane JF, Fletcher CD. Genes Chromosomes and Cancer 2012;51:140-148.
8. Antonescu CR, Zhang L, Chang NE, et al. *EWSR1-POU5F1* fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone and visceral lesions showing common involvement of EWSR1 gene rearrangement. Genes Chromosomes Cancer 2010;49:1114-1124.
9. Gleason BC, Fletcher CD. Myoepithelial carcinoma of soft tissue in children: an aggressive neoplasm analysed in a series of 29 cases. Am J Surg Pathol 2007; 31:1813-1824.
10. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens M (Eds.): WHO classification of tumours of soft tissue and bone. IARC: Lyon 2013.
11. Fletcher CDM eds. Diagnostic histopathology of tumors. 3rd edition, 2007, Elsevier Philadelphia, USA.
12. Rowley JD. A story of swapped ends. Science 2013;340:1412-1413
13. Berman DM, Bosenberg MW, Orwant RL, et al. Investigative pathology: leading the post-genomic revolution. Lab Invest 2012;92:4-8

Chapter 10

Nederlandse Samenvatting

Nederlandse Samenvatting

Gedurende de afgelopen jaren hebben nieuwe moleculaire technieken ons veel inzicht gegeven op het gebied van weke delen tumoren, met name over genetische veranderingen met als gevolg de identificatie van nieuwe entiteiten, herziening van bestaande classificaties en de implementatie van nieuwe diagnostische methoden.

Deze inzichten hebben belangrijke gevolgen gehad voor de pathologie van de weke delen tumoren, en zeker voor de patiënt en diens behandelaar.

Het onderzoek zoals beschreven in dit proefschrift richt zich op een aantal aspecten op het gebied van de weke delen pathologie. Als eerste wordt aandacht gegeven aan de betekenis van gen herrangschikking in weke delen tumoren, met name die, waarbij het EWSR1 gen is betrokken. Volgend onderwerp van aandacht is het diagnostisch belang van tumorsuppressor genen in enkele specifieke weke delen entiteiten, te weten de epithelioide sarcomen and cellulaire angiofibromen en in het laatste hoofdstuk wordt de diagnostische rol van mutaties in het oncogen *CTNNB1* in het ontstaan van desmoid fibromatose op kinderleeftijd onderzocht en geëvalueerd.

EWSR1 gen herrangschikkingen zijn voor het eerst in Ewing sarcomen aangetoond. Uit meer recent onderzoek is gebleken dat herrangschikkingen van dit gen ook bij het ontstaan van andere tumoren een rol spelen. Dit betreft myoepitheliale tumoren, het extraskeletale myxoide chondrosarcoom, de desmoplastische small round cell tumor, het clear cell sarcoom, de clear cell sarcoma-like gastrointestinale tumor, een subgroep binnen de myxoide liposarcomen en het laaggradig fibromyxoid sarcoom/ scleroserende epithelioid fibrosarcoom. Ook in de niet tot de groep van weke delen tumoren behorende epitheliale tumoren zoals het hyaliniserende clear cell carcinoom van de speekselklier en clear cell odontogeen carcinoom zijn *EWSR1* gen herrangschikkingen gevonden.

In dit proefschrift is vooral aandacht geschonken aan de betekenis van de *EWSR1* gen herrangschikking in de myoepitheliale tumoren en hun belang in het kader van de diagnostiek. Hoofdstuk 2 t/m 5 zijn hieraan gewijd.

Myoepitheliale tumoren van de huid, weke delen, bot en andere organen lijken morfologisch op elkaar. Ze kunnen zowel goedaardig als kwaadaardig zijn en het onderscheid tussen beide berust op morfologische criteria. Bovendien zijn deze tumoren genetisch verwant omdat ze in ca. 50% *EWSR1* herrangschikking vertonen, in ieder geval voor de lesies in de weke delen en in de huid. Meerdere fusie genen zijn bekend - *POUF5F1, PBX1, ZNF444,* and *ATF1.* De laatste twee genen tonen de laagste frequentie. *EWSR1-ATF1* werd in een van onze weke delen tumoren gevonden (**hoofdstuk 3**).

Samenvattend blijkt dat (benigne en maligne) myoepitheliale tumoren in verschillende lokalisaties morfologisch en vooral genetisch gerelateerd zijn, met name deze van huid en weke delen. De relatief lage percentage *EWSR1* geherrangschikte casus (ca. 50%) laat de genetische heterogeniteit zien die gepaard gaat met morfologische

diversiteit. Het voorkomen van *EWSR1* rearrangement ook in deze entiteit expandeerd het spectrum van lesies met *EWSR1* rearrangement. *EWSR1-ATF1* fusie is in verschillende benigne en maligne entiteiten aantoonbaar, derhalve is een histopathologische en immuunhistochemische beoordeling voor de correcte diagnose noodzakelijk.

Een maligne weke delen tumor die morfologisch op een myoepitheliale tumor kan lijken en bovendien eveneens *EWSR1* herrangschikking vertoont is het extra-skeletale myxoid chondrosarcoom. De *EWSR1* fusiepartner in deze tumor is *NR4A3*. De consistente aanwezigheid van dit specifieke fusie gen is derhalve een diagnostisch criterium om het onderscheid te maken tussen extraskeletaal myxoid chondrosarcoom en myoepitheliale tumoren. De mogelijkheid om dit onderscheid te maken is belangrijk vanwege een verschil in hun klinisch gedrag; de maligne myoepitheliale tumoren zijn aggressiever (**hoofdstuk 4**).

Een andere morfologische nabootser van myoepitheliale tumoren is de myxoide variant van het epithelioide sarcoom. Aanvullende immuunhistochemie met de marker INI1 helpt het onderscheid te maken, want het INI1 protein is in epithelioide sarcomen consistent afwezig terwijl het in benigne myoepitheliale tumoren wel aanwezig is. Dit konden wij bevestigen in onze analyses van de cutane myoepitheliale tumoren en de epithelioide sarcomen (**hoofdstuk 2 en 5**).

Onderliggende veranderingen in *INI1*, gelocaliseerd op de lange arm van chromosoom 22, zijn deels intragenetisch met deleties en mutaties. Als alternatief worden epigenetische inactiverende veranderingen gediscusseerd. Er is gesteld dat verlies van het eiwit van *INI1* een marker is voor epithelioide sarcomen, maar er moet rekening mee worden gehouden dat INI1 ook afwezig is in een deel van andere epithelioide tumoren zoals myoepitheliale carcinomen en epithelioide maligne perifere zenuwschedetumoren. De genetische achtergrond hiervan is nog onduidelijk. In **hoofdstuk 6** wordt de betekenis van genetische veranderingen voor de diagnostiek van chondroide lipomen besproken. Deze tumoren liggen meestal in de diepe weke delen en zijn sinds kort bekend met de translocatie t(11;16)(q13;p13), leidend tot het ontstaan van het fusiegen *C11orf95-MKL2*. Deze tumoren kunnen vooral vanwege hun chondromyxoide matrix lastig te onderscheiden zijn van maligne weke delen tumoren zoals extraskeletaal myxoid chondrosarcoom en myxoid liposarcoom of, vanwege een overlappend immunoprofiel met positiviteit voor keratine en S100 verward worden met myoepitheliale tumoren.

Deze studie illustreert de meerwaarde van moleculaire technieken bij het maken van het onderscheid tussen morfologisch en immuunhistochemisch overlappende entiteiten. **Hoofdstuk 7** gaat over de deletie van het tumorsuppressorgen *RB1* in cellulaire angiofibromen. Deze recurrente aberratie wordt ook gevonden in de morfologisch gerelateerde tumoren spoelcellipoom en mammary-type myofibro-blastoom. Derhalve kan gespeculeerd worden dat deze benigne lesies een spectrum van een entiteit representeren. Moleculaire technieken kunnen dus niet alleen

een rol spelen bij het onderscheiden van verschillende entiteiten, maar ook een verwantschap tussen ogenschijnlijk verschillende tumorsoorten onthullen.

Hoofdstuk 8 is gewijd aan de desmoid type fibromatosen in het hoofd hals gebied bij kinderen beschreven. Deze locaal aggressieve tumoren komen bij kinderen in deze regio vaker voor en tonen meestal een betere prognose vergeleken met volwassenen. Zij zijn gekenmerkt door mutaties in het *CTNNB1* gen. Uit ons onderzoek is gebleken dat desmoiden in het hoofd hals gebied een grotere variatie in mutaties in dit gen laten zien dan elders in het lichaam het geval is. Bovendien vonden we een nieuwe en een nog slechts éénmaal tevoren beschreven mutatie in deze patiëntengroep. Ook zagen we in deze patiëntengroep een desmoplastisch fibroom, een lesie morfologisch identiek aan desmoid maar in het bot gelokaliseerd, met een voor desmoid klassieke mutatie. Dit ondersteunt de tot op heden controversiële opvatting dat het desmoplastisch fibroom in feite een intraossaal desmoid is.

De laatste twee hoofdstukken laten zien dat implementatie van genetische characteristieken van weke delen tumoren de morfologische classificaties kunnen beinfloeden in die zin dat entiteiten worden gevormd waarin morfologisch op elkaar lijkende lesies ook een gezamelijke genetische achtergrond hebben.

Blik op de toekomst

Zoals tot op heden ook benoemd in de recente WHO classificatie van weke delen en bot is microscopie nog steeds de gouden diagnostiek standaard, soms aangevuld met immunohistochemie en moleculaire analyses. In principe wordt een tumor gedefineerd door de veronderstelde cel van herkomst en de graad van differentiatie. Deze robuuste en betrouwbare methoden voldoen nog steeds heel goed en leiden nog steeds tot het definieren van nieuwe entiteiten.

De classificatie van tumoren berust op het onderkennen van zogenaamde entiteiten. Een entiteit is een ziekte die wordt gedefinieerd door morfologische, immunohistochemische, genetische en klinische karakteristieken. Idealiter worden in deze genoemde karakteristieken voor elke entiteit specifieke criteria gevonden die helpen prognose en behandeling te bepalen wat zowel voor de patiënt als voor de clinicus van belang is.

In de laatste jaren heeft de genetische karakerisering van weke delen tumoren grote vlucht genomen. Deze detectie kan behulpzaam zijn bij het begrijpen van de pathobiologie van een lesie, daaronder begrepen de bij tumorontwikkeling betrokken cellulaire metabole routes alsook identificeren van nieuwe diagnostische markers (bv. gen mutaties of amplificaties en fusie genen). In de praktijk methoden zijn PCR en sequencing (Sangermethode, gene scanning and next generation sequencing) en voorts fluorescentie in situ hybridisatie. Door deze ontwikkelingen zal de diagnostiek

op het gebied van weke delen tumoren veranderen. Op de voorgrond staat dan de genetische karakterisering in plaats van het bepalen van de tumorentiteit aan de hand van cel van origine en graad van differentiatie. Dit verschaft de informatie die noodzakelijk is om met doelgerichte therapie in te grijpen op het afwijkende metabolisme van de tumorcel, de zogenaamde “targeted treatment”.

Wat zijn de consequenties van dit palet aan genetische technieken in de diagnostiek van weke delen tumoren? Het is te verwachten dat next generation sequencing een hogere incidentie van specifieke genetische afwijkingen per tumor of entiteit toont dan wat tot nu toe is gevonden. Daardoor kan de tumorclassificatie worden verbeterd, zoals ook in dit proefschrift is aangetoond. Nog belangrijker is de vraag welke genetische veranderingen leiden tot veranderde gen functie en zijn derhalve relevant voor de tumorgroei? Om deze vraag te beantwoorden is weefselonderzoek op RNA niveau en eiwit niveau noodzakelijk. De uit derglijk onderzoek verkregen data zullen worden gecorreleerd aan de klinische gegevens om een eventuele relatie met klinisch beloop te achterhalen. Op deze wijze zijn al diverse genen en metabole routes gevonden als aangrijpingspunt voor doelgerichte behandeling en reeds goedgekeurd voor de therapie van andere tumoren maar of deze ook van belang zijn voor de behandeling van sarcomen, is nog niet voldoende onderzocht. Bovendien is het redelijk te veronderstellen dat er in de toekomst meer genetische veranderingen met therapeutisch belang worden gevonden waardoor de behandeling van weke delen tumoren en met name van de sarcomen zal veranderen.

In de toekomst zal de diagnostische aanpak worden uitgebreid door het in kart brengen van de moleculaire basis van de ziektes met als gevolg doelgerichte interventie. Centraal in dit radicaal veranderde model van patiëntenzorg staat de toepassing van genomische technologiën waarmee in korte tijd grote aantallen gegevens kunnen worden verkregen. Wat de eerstkomende jaren niet zal veranderen is het belang van de histomorphologie omdat weefselkarakterisatie onmisbaar blijft voor het vaststellen van representativiteit van het onderzochte materiaal en om onverwachte (secundaire of reactieve) lesies uit te sluiten. Bovendien kunnen in verschillende tumoren identieke genetische afwijkingen voorkomen, wat histologische beoordeling van de lesies voor therapiekeuze essentieel maakt.

Pathologen hebben een belangrijke functie in deze weefselanalyse. Zij zijn ervoor verantwoordelijk genetische en moleculaire informaties in de morfologische context te plaatsen waarbij ook kennis van de interacties van cellen, genen en micromilieu niet gemist kan worden. Om de clinici de voor een goed beleid noodzakelijke informatie te geven is een goede samenwerking van de pathologen met moleculaire genetici en bioinformatici nodig. Uit bovenstaande overwegingen is af te leiden dat onderzoek van het genoom essentieel is in het verkrijgen van een tumorclassificatie die niet alleen op morfologische maar ook op moleculaire data is gebaseerd. De beschikbaarheid van weefsels van goede kwaliteit is hiervoor van groot belang. Verder

dient te worden benadrukt dat een definitieve validatie van biomarkers is afhankelijk van patiënten die participeren in prospectieve klinische studies. Verdere ontwikkeling van de moleculaire diagnostiek staat of valt derhalve met het beschikbaar zijn van tumormateriaal van goede kwaliteit, afkomstig van adequaat gedocumenteerde en gevolgde groepen patiënten. De belangrijke rol van de patholoog in dit verband is al eerder genoemd. Hun expertise in weefselkarakterisatie is in deze studies onontbeerlijk.

Moleculaire technieken zullen in de toekomst diagnose en behandeling van ziekten verbeteren. De beschikbaarheid van moderne technieken is echter niet hetzelfde als herkennen en begrijpen van een ziekte met als gevolg betere karakterisering van deze ziekte.

Voor de ontwikkeling van targeted therapie bij verschillende cancers hebben we ook meer kennis nodig betreffende tumor specifieke antigene en epigenetische veranderingen. Het doel van individuele geneeskunde is dan ook het opsporen van alle genetische en epigenetische (bv. DNA methylering en histon modificatie) veranderingen die een rol spelen in tumorontwikkeling of tumorprogressie en behandelbaar zijn. Deze innovaties zullen mogelijk onverwachte resultaten opleveren en de huidige zienswijze ten aanzien van weke delen tumoren veranderen. Het door al dit onderzoek beoogde doel verandert evenwel niet: optimale behandeling van de ziekte en bevorderen van het welzijn van de patiënt.

Conclusie

Vanwege de huidige beschikbaarheid van tumorspecifieke behandelopties van een tumor is het nu nog meer dan voorheen belangrijk de juiste diagnose te stellen. Moleculaire diagnostiek speelt hierbij in toenemende mate een essentiële rol.

Om te komen tot deze juiste diagnose en een optimale behandeling is het belangrijk om zowel de klinische als de radiologische, histologische en moleculair biologische aspecten in de overwegingen te betrekken.

Appendices

Curriculum vitae
List of publications
Acknowledgements

Curriculum vitae

Uta Flucke werd geboren op 16 augustus 1967 te Heiligenstadt, Duitsland. Zij volgte een opleiding als apothekersassistente en behaalde het “Abitur” (VWO-diploma) 1988. Daarna ging ze een semester scheikunde studeren voordat ze naar Westduitsland verhuisde. Van 1989 -1996 studeerde ze geneeskunde in Keulen en ze begon daarna met haar opleiding tot patholoog met twee jaar onderbreking op de afdeling heelkunde van het UMC Keulen. De opleiding tot patholoog werd 2004 in Aken afgerond. Zij ging toen werken in het Universitaire Ziekenhuis in Bonn. Vanaf oktober 2007 werkt zij als patholoog met specialisatie weke delen en bot tumoren in het Radboud UMC waar ook haar proefschrift is tot stand gekomen.

List of publications

1. Thiele J, Zirbes TK, Lorenzen J, Kvasnicka HM, Scholz S, Erdmann A, Flucke U, Diehl V, Fischer R. Hematopoietic turnover index in reactive and neoplastic bone marrow lesions: quantification by apoptosis and PCNA labeling. Ann Hematol 1997;75:33-39.
2. Thiele J, Kvasnicka HM, Zirbes TK, Flucke U, Niederle N, Leder LD, Diehl V, Fischer. Impact of clinical and morphological variables in classification and regression tree-based survival (CART) analysis of CML with special emphasis on dynamic features. R. Eur J Haematol 1998;60:35-46.
3. Thiele J, Wickenhauser C, Neuwirth C, Schulze HJ, Flucke U, Kvasnicka HM, Borchmann P, Krech R, Fischer R. Effect of IFN-alpha on normal human hematopoiesis: an immunohistochemical and morphometric study on trephine biopsy specimens. J Interferon Cytokine Res 1998;18:247-253.
4. Baldus SE, Zirbes TK, Weidner IC, Flucke U, Dittmar E, Thiele J, Dienes HP.Comparative quantitative analysis of macrophage populations defined by CD68 and carbohydrate antigens in normal and pathologically alterated human liver tissue. Anal Cell Pathol 1998;16:141-150.
5. Hess S, Smola H, Sandaradura De Silva U, Hadaschik D, Kube D, Baldus SE, Flucke U, Pfister H. Loss of IL-6 receptor expression in cervical carcinoma cells inhibits autocrine IL-6 stimulation: abrogation of constitutive monocyte chemoattractant protein-1 production. J Immunol 2000;15:1939-1948.
6. Baldus SE, Zirbes TK, Weingarten M, Fromm S, Glossmann J, Hanisch FG, Mönig SP, Schröder W, Flucke U, Thiele J, Hölscher AH, Dienes HP. Increased galectin-3 expression in gastric cancer: correlations with histopathological subtypes, galactosylated antigens and tumor cell proliferation. Tumour Biol 2000;21:258-266.
7. Thiele J, Kvasnicka HM, Beelen DW, Flucke U, Spoer C, Paperno S, Leder LD, Schaefer UW. Megakaryopoiesis and myelofibrosis in chronic myeloid leukemia after allogenic bone marrow transplantation: an immunohistochemical study of 127 patients. Mod Pathol 2001;14:129-138.
8. Flucke U, Zirbes TK, Schröder W, Mönig SP, Koch V, Schmitz K, Thiele J, Dienes HP, Hölscher AH, Baldus SE. Expression of mucin-associated carbohydrate core antigens in esophageal squamous cell carcinomas. Anticancer Res 2001;21:2189-2193.
9. Baldus SE, Zirbes TK, Glossmann J, Fromm S, Hanisch FG, Mönig SP, Schröder W, Schneider PM, Flucke U, Karsten U, Thiele J, Hölscher AH, Dienes HP. Immunoreactivity of monoclonal antibody BW835 represents a marker of progression and prognosis in early gastric cancer. Oncology 2001;61:147-155.
10. Baldus SE, Hanisch FG, Pütz C, Flucke U, Mönig SP, Schneider PM, Thiele J, Hölscher AH, Dienes HP. Immunoreactivity of Lewis blood group and mucin peptide core antigens: correlations with grade of dysplasia and malignant tranformation in the colorectal adenoma-carcinoma sequence. Histol Histopathol 2002;17:191-198.
11. Flucke U, Mönig SP, Baldus SE, Zirnes TK, Bollschweiler E, Thiele J, Dienes HP, Hölscher AH. Differences between biopsy- or specimen-related Laurén and World Health Organization classification in gastric cancer. World J Surg 2002;26:137-140.
12. Baldus SE, Mönig SP, Hanisch FG, Zirbes TK, Flucke U, Oelert S, Zilkens G, Madejczik B, Thiele J, Schneider PM, Hölscher AH, Dienes HP. Comparative evaluation of the prognostic value of MUC1, MUC2, sialyl-Lewis(a) and sialyl-Lewis(x) antigens in colorectal adenocarcinoma. Histopathology 2002;40:440-449.
13. Flucke U, Steinborn E, Dries V, Mönig SP, Schneider PM, Thiele J, Hölscher AH, Dienes HP, Baldus SE. Immunoreactivity of cytokeratins (CK7, CK 20) and mucin peptide core antigens (MUC1, MUC2, MUC5AC) in adenocarcinomas, normal and metaplastic tissues of the distal oesophagus, oesophago-gastric junction and proximal stomach. Histopathology 2003;43:127-134.
14. Rhiem K, Flucke U, Schmutzler RK. BRCA1-associated breast carcinomas frequently present with benign sonographic features. AJR Am J Roentgenol 2006;186:E11-2.
15. Rhiem K, Flucke U, Engel C, Wappenschmidt B, Reinecke-Lütghe A, Büttner R, Schmutzler RK. Association of the BRCA1 missense variant R1699W with a malignant phylloides tumor of the breast. Cancer Genet Cytogenet 2007;176:76-79.

16. Braun M, Pölcher M, Schrading S, Zivanovic O, Kowalski T, Flucke U, Leutner C, Park-Simon TW, Rudlowski C, Kuhn W, Kuhl CK. Influence of preoperative MRI on the surgical management of patients with operable breast cancer. Breast Cancer Res Treat 2008;111:179-187.
17. Braun M, Flucke U, Debald M, Walgenbach-Bruenagel G, Walgenbach KJ, Höller T, Pölcher M, Wolfgarten M, Sauerwald A, Keyver-Paik M, Kühr M, Büttner R, Kuhn W. Detection of lymphovascular invasion in early breast cancer by D2-40 (podoplanin): a clinically useful predictor for axillary lymph node metastases. Breast Cancer Res Treat 2008;112:503-511.
18. Fischer HP, Flucke U, Zhou H. Pathology along the liver sinusoids: endothelial and perisinusoidal findings. Pathologe 2008;29:37-46.
19. Flucke U, Fischer HP. Pathology along the liver sinusoids: intrasinusoidal findings. Pathologe 2008; 29:27-36.
20. Van Laarhoven HW, Vinken M, Mus R, Flucke U, Oyen WJ, van der Graaf WT. The diagnostic hurdle of an elderly male with bone pain: how 18F-FDG-PET led to the diagnosis of a leiomyosarcoma of the adrenal gland. Anticancer Res 2009;29:469-472.
21. Flucke U, Slootweg PJ, Mentzel T, Pauwels P, Hulsebos T. Re: Infrequent SMARB1/INI1 gene alteration in epitheloid sarcoma: a useful tool in distinguishing epitheloid sarcoma from malignant rhabdoid tumor: direct evidence of mutational inactivation of SMARB1/INI1 in epitheloid sarcoma. Hum Pathol 2009;40:1361-1362.
22. Debald M, Pölcher M, Flucke U, Walgenbach-Brünagel G, Walgenbach KJ, Höller T, Wolfgarten M, Rudlowski C, Büttner R, Schild H, Kuhn W, Braun M. Increased detection of lymphatic vessel invasion by D2-40 (podoplanin) in early breast cancer: possible influence on patient selection for accelerated partial breast irradiation. Int J Radiat Oncol Biol Phys 2010;77:1128-1133.
23. Van de Luijtgaarden AC, Flucke UE, van der Graaf WT, de Rooy JW, Bonenkamp HJ, de Geus-Oei LF. Malignant transformation in an area with elevated F-18 FDG uptake within a low metabolic benign neurofibroma. Clin Nucl Med 2010;35:271-272.
24. Jongmans MC, Hoogerbrugge PM, Hilkens L, Flucke U, van der Burgt I, Noordam K, Ruiterkamp-Versteeg M, Yntema HG, Nillesen WM, Ligtenberg MJ, van Kessel AG, Kuiper RP, Hoogerbrugge N. Noonan syndrome, the SOS1 gene and embryonal rhabdomyosarcoma. Genes Chromosomes Cancer 2010;49:635-641.
25. van de Luijtgaarden A, van der Ven A, Leenders W, Kaal S, Flucke U, Oyen W, van der Graaf W. Imaging of HIV-associated Kaposi sarcoma; F-18-FDG-PET/CT and In-111-bevacizumabscintigraphy. J Acquir Immune Defic Syndr 2010;54:444-446.
26. Flucke U, Flucke MT, Hoy L, Breuer E, Goebbels R, Rhiem K, Schmutzler R, Winzenried H, Braun M, Steiner S, Buettner R, Gevensleben H. Distinguishing medullary carcinoma of the breast from high-grade hormone receptor-negative invasive ductal carcinoma: an immunohistochemical approach. Histopathology 2010;56:852-859.
27. Hagleitner MM, Coenen MJ, Jeuken JW, Flucke U, Schreuder HW, te Loo DM, Hoogerbrugge PM. Taqman genotyping assays can be used on decalcified and paraffin-embedded tissue from patients with osteosarcoma. Pediatr Blood Cancer 2011;56:35-38.
28. Flucke U, van Krieken JH, Mentzel T. Cellular angiofibroma: analysis of 25 cases emphasizing its relationship to spindle cell lipoma and mammary-type myofibroblastoma. Mod Pathol 2011 Jan;24(1):82-89.
29. Vriens D, de Geus-Oei LF, Flucke UE, van der Kogel AJ, Oyen WJ, Vierhout ME, van der Meer JW. Benign uterine uptake of FDG: a case report and review of literature. Neth J Med 2010;68:379-380.
30. Flucke U, Hulsebos TJ, van Krieken JH, Mentzel T. Myxoid epithelioid sarcoma: a diagnostic challenge. A report on six cases. Histopathology. 2010 Nov;57(5):753-759.
31. van den Brand M, Flucke UE, Bult P, Weemaes CM, Deuren M. Angiosarcoma in a patiënt with immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome. Am J Med Genet 2011;155A:622-625.
32. de Rooy JW, Hambrock T, Vriens D, Flucke UE, van der Geest IC, van de Luijtgaarden AC, Schreuder BW, de Geus-Oei LF. F-18 FDG PET/CT as a crucial guide toward optimal treatment planning in a case of postiiradiation sarcoma 10 years after primary bone lymphoma of the pelvis. Clin Nucl Med 2011;36:565-567.

33. van der Wal WA, Unal H, de Rooy JW, Flucke U, Veth RP. Fibrous dysplasia of bone associated with soft-tissue myxomas as well as an intraosseous myxoma in a woman with Mazabraud's syndrome: a case report. J Med Case Rep 2011;5:239.
34. Flucke U, Palmedo G, Blankenhorn N, Slootweg PJ, Kutzner H, Mentzel T. *EWSR1* gene rearrangement occurs in a subset of cutaneous myoepithelial tumors: a study of 18 cases. Mod Pathol 2011 Nov;24(11):1444-50.
35. Hagleitner MM, Hoogerbrugge PM, van der Graaf WT, Flucke U, Schreuder HW, te Loo DM. Age as prognostic factor in patients with osteosarcoma. Bone 2011;49:1173-1177.
36. Van Asseldonk EJ, de Sévaux RG, Flucke UE, de Rooy JW, Netea-Maier RT, Timmers HJ. Multiple osteolytic bone lesions. Neth J Med 2011;69:399,403.
37. Flucke U, Mentzel T, Verdijk MA, Slootweg PJ, Creytens DH, Suurmeijer AJ, Tops BB. *EWSR1-ATF1* chimeric transcript in a myoepithelial tumor of soft tissue: a case report. Hum Pathol 2011 May; 43(5):764-8.
38. van Gaal JC, Flucke UE, Roeffen MH, de Bont ES, Sleijfer S, Mavinkurve-Groothuis AM, Suurmeijer AJ, van der Graaf WT, Versleijen-Jonkers YM. Anaplastic lymphoma kinase aberrations in rhabdomyosarcoma: clinical and prognostic implications. J Clin Oncol 2012 Jan 20;30(3):308-15.
39. Flucke U, Tops BB, Verdijk MA, van Cleef PJ, van Zwam PH, Slootweg PJ, Bovée JV, Riedl RG, Creytens DH, Suurmeijer AJ, Mentzel T. NR4A3 rearrangement reliably distinguishes between the clinicopathologically overlapping entities myoepithelial carcinoma of soft tissue and cellular extraskeletal myxoid chondrosarcoma. Virchows Arch 2012 Jun;460(6):621-628.
40. Van Gaal JC, Van Der Graaf WT, Rikhof B, Van Hoesel QG, Teerenstra S, Suurmeijer AJ, Flucke UE, Loeffen JL, Sleijfer S, De Bont ES. The impact of age on outcome of embryonal and alveolar rhabdomyosarcoma patients. A multicenter study. Anticancer Res 2012;32:4485-4497.
41. van de Luijtgaarden AC, Versleijen-Jonkers YM, Roeffen MH, Schreuder HW, Flucke UE, van der Graaf WT. Prognostic and therapeutic relevance of the IGF pathway in Ewing's sarcoma patients. Target Oncol 2013;8:253-60.
42. Vlenterie M, Flucke U, Hofbauer LC, Timmers HJ, Gastmeier J, Aust DE, van der Graaf WT, Wesseling P, Eisenhofer G, Lenders JW. Pheochromocytoma and gastrointestinal stromal tumors in patients with neurofibromatosis type I. Am J Med 2013;126:174-180.
43. Fleuren ED, Roeffen MH, Leenders WP, Flucke UE, Vlenterie M, Schreuder HW, Boerman OC, van der Graaf WT, Versleijen-Jonkers YM. Expression and clinical relevance of MET and ALK in Ewing sarcomas. Int J Cancer 2013;133:427-436.
44. Flucke U, Tops BB, de Saint Aubain Somerhausen N, Bras J, Creytens DH, Küsters B, Groenen PJ, Verdijk MA, Suurmeijer AJ, Mentzel T. Presence of C11orf95-MKL2 is a consistent finding in chondroid lipomas: a study of 8 cases. Histopathology 2013;62:925-30.
45. Van Gaal JC, Roeffen MH, Flucke UE, van der Laak JA, van der Heijden G, de Bont ES, Suurmeijer AJ, Versleijen-Jonkers YM, van der Graaf WT. Simultaneous targeting of insulin-like growth factor-1 receptor and anaplastic lymphoma kinase in embryonal and alveolar rhabdomyosarcoma: a rational choice. Eur J Cancer 2013;49:3462-70.
46. Flucke U, Requena L, Mentzel T. Radiation-induced vascular lesions of the skin: an overview. Adv Anat Pathol 2013;20-407-15.
47. van de Luijtgaarden AC, Roeffen MH, Leus MA, Flucke UE, Schreuder BH, van der Graaf WT, Versleijen-Jonkers YM. IGF signaling pathway analysis of osteosarcomas reveals the prognostic value of pAKT localization. Future Oncol 2013;9:1733-40.
48. Hulsebos T, Kenter S, Siebers-Renelt U, Hans V, Wesseling P, Flucke U. SMARCB1 involvement in the development of leiomyoma in a patient with schwannomatosis. Am J Surg Pathol 2014;38:421-425.
49. Flucke U, Tops BB, van Diest P, Slootweg PJ. Desmoid-type fibromatosis of the head and neck region in the pediatric population. A clinicopathological and genetic study of 6 cases. Histopathology 2014;64:769-776..
50. Bekers EM, van Engen-van Grunsven AC, Groenen PJ, Westdorp H, Koornstra RH, Bonenkamp JJ, Flucke U, Blokx WA. Metastatic melanoma mimicking solitary fibrous tumor: report of two cases. Virchows Arch 2014;464:247-251.

51. Pol FJ, Flucke U, Jafari K. Intrathoracic hibernoma. Ned Tijdschr Geneeskd 2014;158:A6610.
52. Van Geel MJ, Wijnen M, Hoppenreijs EP, Gierenz N, Spruijt L, van Koolwijk MP, Flucke U, Blokx WA, Seyger MM. Hypertrophic left calf and multiple flesh-colored subcutaneous tumours in a 5-year-old girl. Acta Derm Venerol 2014;94:619-622.
53. Hulsbos TJ, Kenter S, Verhagen WI, Baas F, Flucke U, Wesseling P. Premature termination of SMARCB1 translation may be followed by reinitiation in schwannomatosis-associated schwannomas, but results in absence of SMARCB1 expression in rhabdoid tumors. Acta Neuropathol 2014;128:439-448.
54. Flucke U, Vogels RJ, de Saint Aubain Somerhausen N, Creytens DH, Riedl RG, van Gorp JM, Milne AN, Huysentruyt CJ, Verdijk MA, van Asseldonk MM, Suurmeijer AJ, Bras J, Palmedo G, Groenen PJ, Mentzel T. Epithelioid hemangioendothelioma: clinicopathologic, immunohistochemical, and molecular analysis of 39 cases. Diagn Pathol 2014;9:131.
55. Fleuren ED, Versleijen-Jonkers YM, Roeffen MH, Franssen GM, Flucke UE, Houghton PJ, Oyen WJ, Boerman OC, van der Graaf WT. Temsirolimus combined with cisplatin or bevacizumab is active in osteosarcoma models. Int J Cancer 2014

Dankwoord

De inzet en steun van vele mensen waren onmisbare voorwaarden voor het tot stand komen van dit proefschrift.

Prof. dr. Han van Krieken (promotor), beste Han, jij en de staf van de afdeling pathologie hebben ervoor gezorgd dat ik hier mocht komen werken. Jullie hebben me welkom geheten en me veel steun gegeven zowel voor mijn werk als patholoog als ook voor mijn onderzoek. Er was vertrouwen in mij als mens, patholoog en onderzoekster. Dat heeft me gemotiveerd dit onderzoek te doen. Ik heb veel van je geleerd met name van je manier van onderzoek benaderen en van je ervaring. Ik wil je van harte bedanken.

Prof. dr. Piet Slootweg (promotor), beste Piet, met jou heb ik altijd heel nauw samen gewerkt. Jij hebt heel veel geduld met mij gehad en me altijd gesteund. Ik had veel plezier met het werk wat we gezamenlijk hebben verzet en vooral mocht ik heel veel van je klinische ervaring leren. Hartelijk dank voor je optimisme en je constructieve kritiek.

Prof. dr. Winette van der Graaf (promotor), beste Winnette, jij bent de sarcoom specialist in het RUMC en hebt internationaal naam gemaakt op het gebied van weke delen tumoren. Onze gezamenlijke patientenzorg levert inspiraties voor ons onderzoek op. Jij staat altijd open voor wetenschappelijke samenwerking en geeft enorme steun ook met je internationale ervaring en netwerk. Ik hoop dat onze samenwerking ook in de toekomst vruchtbaar zal zijn. Heel erg bedankt.

De leden van de manuscriptcommissie, **prof. Geurts van Kessel**, **prof. Pauwels** en **prof. de Wilt**, wil ik hartelijk bedanken voor het kritisch doornemen en beoordelen van dit proefschrift.

Dr. Yvonne Versleijen, beste Yvonne, de samenwerking met jou was vanaf het begin heel leuk en vruchtbaar. We hebben altijd goede discussies gehad en jij was altijd open en benaderbaar. Ik stel onze samenwerking heel erg op prijs en hoop dat we daar nog vele leuke projecten van kunnen maken. Dank voor je hulp en je positieve insteek.

Dr. Bastiaan Tops, beste Bastiaan, jij was de meest flexible adviesgever voor mij. Jouw steun was met name in het begin van mijn onderzoek heel waardevol. Veel dank voor de tijd die je hebt vrijgemaakt, je adviezen en je inzet. Gelukkig ben je nu moleculair bioloog hier in huis en zodoende is onze samenwerking ook voor de toekomst gewaarborgd.

Dr. P. Groenen, beste Patricia, dank voor je inzet met je kennis als moleculair bioloog. Jij hebt heel veel moleculair werk samen met **Marian Verdijk** verzet. Zonder jullie inzet was het praktische werk en daarmee het proefschrift niet van de grond gekomen. Ook veel dank aan alle analisten op de diverse laboratoria voor de werkzaamheden die mede tot dit proefschrift hebben geleid.

Prof. dr. Thomas Mentzel, lieber Thomas, mit Dir habe ich die Begeisterung für Weichgewebstumoren teilen dürfen. Die Zusammenarbeit war immer ohne Vorbehalt und hat mich sehr motiviert. Es hat ungeheuren Spass gemacht und ich habe sehr viel gelernt. Ich würde mich sehr freuen, wenn wir diese freunschaftliche Zusammenarbeit fortsetzen können.

Prof. dr. Pauwels, beste Patrick, met jou hulp heb ik de eerste stappen op het weke delen terrein in Nederland mogen zetten. Jij hebt me aangemoedigd om door te gaan met onderzoek op het gebied van weke delen tumoren. Veel dank ervoor.

Dr. Theo Hulsebos, beste Theo, jij hebt het moleculaire werk van ons INI1 project gedaan. Dat was voor mij een heel goed begin van het onderzoek hier in Nijmegen. Hartelijk dank ook voor goede discussies en de leuke samenwerking, waaruit verdere resultaten zijn ontstaan.

Beste pathologie stafleden, ook door jullie hulp en steun was er ruimte om aan dit proefschrift te werken. Hartelijk dank ervoor.

Beste leden van de sarcomenwerkgroep, de samenwerking met jullie geeft me vertrouwen in mijn werk en inspiraties voor onderzoek. Jullie allen van harte bedankt.

Liebe Eltern, liebe Schwestern, liebe Familie und liebe Freunde, ohne Eure liebevolle Unterstützung wäre diese Arbeit nicht entstanden. Tausend Dank für alles.